1982

cancer treatment reports

September 1982
Volume 66
Number 9
Pages 1687-1789

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES Public Health Service National Institutes of Health

cancer treatment reports

National Cancer Institute, Vincent T. DeVita, Jr., ***Director***
Division of Cancer Treatment, Bruce A. Chabner, ***Director***
Scientific Information Branch, Susan Molloy Hubbard, ***Chief***

Editorial Board

John S. Macdonald, ***Editor-In-Chief***

Associate Editors

Martin D. Abeloff
Joseph Aisner
Lewis Aronow
Ross C. Donehower
Daniel C. Ihde
Allen S. Lichter
Robert W. Makuch
David A. Pistenmaa
Philip A. Pizzo
Charles A. Schiffer
Mary K. Wolpert
Paul V. Woolley

Advisory Board

Gianni Bonadonna
George P. Canellos
Richard H. Creech
Harvey M. Golomb
Robert L. Goodman
Stephen B. Howell
Randall K. Johnson
Irwin H. Krakoff
Brigid G. Leventhal
Robert B. Livingston
Albert F. LoBuglio
William L. McGuire
Franco M. Muggia
Carol S. Portlock
Sydney E. Salmon

Editorial Staff

Jean Griffin Baum, ***Managing Editor***
Dee Kittner LeRoy, ***Assistant Managing Editor***

Editors

Elaine Price Beck
Anne McCarthy
Marian Sharp McGrath

Editorial Assistants

Elaine Brown
Susan Lee Gordon
Jana Johnston
Barbara Lineberry

Editorial Policy

The Editorial Board of *Cancer Treatment Reports* will consider manuscripts on original observations in laboratory and clinical research from all scientists. A manuscript is considered for publication with the understanding that it has not been published previously and is submitted exclusively to *Cancer Treatment Reports*. Opinions expressed by the authors are not necessarily those of the publisher or its editors. Scientific contributions in any of the following areas of investigation are solicited: surgery, radiotherapy, chemotherapy, immunotherapy, supportive care, pharmacology, cell biology and kinetics, mechanisms of drug action, and medicinal chemistry.

Submission of Manuscripts

Manuscripts and all inquiries concerning the status of manuscripts should be addressed to: Managing Editor, Cancer Treatment Reports, National Cancer Institute, 7910 Woodmont Ave. Rm 8C08, Landow Bldg, Bethesda, MD 20814 (301-496-6975). All material submitted for consideration in the journal will be subject to review when appropriate by at least two outside reviewers and one member of the Editorial Board. The decision to publish a manuscript is solely the responsibility of the Editorial Board. Prospective authors should consult the "Instructions to Authors," which appears in the second page of every issue.

Subscriptions

Cancer Treatment Reports is for sale by the Superintendent of Documents, US Government Printing Office, Washington, DC 20402. The yearly subscription rate is $47.00 domestic, with an additional $11.75 for foreign mailing. The prices vary for individual issues. Payment is required in advance; check or money order should be made payable to the Superintendent of Documents. Please specify List ID "CAN" (the identifying code for this journal) when requesting a subscription.

INSTRUCTIONS TO AUTHORS

Title Page
The complete title of the manuscript and the full name, affiliation, and address of each author should be included on the title page. Indicate full name and address of author to whom correspondence and/or reprint requests are to be sent.

Summary
Begin the article with a short summary.

Text
Submit an original and 3 copies of the manuscript, typed double-spaced. Use accepted spelling, abbreviation, and hyphenation found in *Webster's New International Dictionary*. Footnotes should be typed at the bottom of the page on which they appear.

Tables
Submit each table on a separate page, typed double-spaced. Please use standard abbreviations; otherwise spell out.

References
Submit references typed double-spaced. Follow the standard form of *Index Medicus*: author, title, name of journal, volume number, page or inclusive pages, and year. In book references give author, title of book, edition (if more than one), city and name of publisher, date of book, volume number, and pages cited. References in text should begin with number 1 and continue consecutively. References to papers presented at meetings should include author; title of paper; title, city, and date of meeting; city and name of publisher; date of publication; volume number; and pages. Papers presented at meetings but not published should be cited in a footnote rather than in the reference section.

Illustrations
Photographs and graphs preferably should be clear, glossy prints. Original art work should be done in black ink for good reproduction. Please send 2 copies of each print. Color photographs cannot be used.

Nomenclature
Names of compounds should conform to American usage. See *USAN and the USP Dictionary of Drug Names* or *The Merck Index* for proper nomenclature.

Brief Reports
Manuscripts published in the Brief Reports section can be no longer than 5 double-spaced pages including references, tables, and a short summary; figures are usually omitted.

Letters to the Editor
Letters to the Editor can be no longer than 3 double-spaced text pages and can be submitted with no more than 1 table and 5 references; figures are usually omitted.

Clinical Trials Summaries
Manuscripts published in this section can be no longer than 300 words and should be submitted with no more than 1 table and 5 references, and no figures. A second table will be permitted only when requested by the editorial board. Tables should include the following: *patient characteristics and prognostic factors* (age/sex, performance status, stage of disease, pathologic types of disease, prior therapy), *drug regimen*, or *standardized toxicity grades* to demonstrate that an appropriate, biologically active dose was administered.

Reprints
Supplied free of charge: 250 reprints (50 reprints for Letters to the Editor) will be sent to the senior author about 3 weeks after publication of the article.

The Secretary of Health and Human Services has determined that the publication of this periodical is necessary in the transaction of the public business required by law of this Department. Use of funds for printing this periodical has been approved by the Director of the Office of Management and Budget through June 30, 1983.

Contents

Cancer Treatment Reports
Volume 66, Number 9
September 1982

Current Controversies in Cancer Management

1687 Chemotherapy for Osteogenic Sarcoma: An Investigative Method, Not a Recipe. **G. Rosen and A. Nirenberg.** Memorial Sloan-Kettering Cancer Center, New York, New York

1699 Is It Ethical Not To Conduct a Prospectively Controlled Trial of Adjuvant Chemotherapy in Osteosarcoma. **B. Lange and A. S. Levine.** The Children's Hospital of Philadelphia, Pennsylvania

Special Feature

1705 The Development of New Cancer Drugs: A Joint Venture in Public Policy by the National Cancer Institute and Food and Drug Administration. **J. R. Crout.** Bureau of Drugs, Food and Drug Administration, Rockville, Maryland

Clinical Studies

1709 Vindesine: An Effective Agent in the Treatment of Non-Small Cell Lung Cancer. **B. E. Furnas, S. D. Williams, L. H. Einhorn, and M. A. Cobleigh.** Indiana University Medical Center, and the Indianapolis Veteran's Administration Medical Center

1713 Studies of Baker's Antifol, Methotrexate, and Razoxane in Advanced Gastric Cancer: A Gastrointestinal Tumor Study Group Report. **H. W. Bruckner, J. J. Lokich, and D. M. Stablein.** Gastrointestinal Tumor Study Group

1719 Epileptic Seizures Associated With Cisplatin Administration. **G. M. Mead, A. M. Arnold, J. A. Green, F. R. Macbeth, C. J. Williams, and J. M. Whitehouse.** Southampton General Hospital, England

Phase I–II Trials

1723 High-Dose Allopurinol Modulation of 5-FU Toxicity: Phase I Trial of an Outpatient Dose Schedule. **T. N. Campbell, S. B. Howell, C. Pfeifle, and B. A. House.** Kaiser Permanente Medical Center, San Diego, California

1729 Phase II Study of Vindesine in Patients With Advanced Breast Cancer. **B. K. Walker, P. C. Raich, J. Fontana, V. P. Subramanian, J. S. Rogers, II, J. A. Knost, and B. Denning.** West Virginia University School of Medicine, Morgantown

Clinical and Preclinical Pharmacology

1733 Pharmacokinetics of Methotrexate and 7-Hydroxymethotrexate Following Infusions of High-Dose Methotrexate. **H. Breithaupt and E. Küenzlen.** Medizinische Klinik der Justus Liebig-Universität, Giessen, Federal Republic of Germany

1743 Dose-Response Curves for Predicting Misonidazole-Induced Peripheral Neuropathy. **J. G. Schwade, R. W. Makuch, J. M. Strong, and E. Glatstein.** National Cancer Institute, Bethesda, Maryland

1751 Metabolism and Mechanism of the Antileukemic Action of Isomeric Aryldimethyltriazenes. **G. Sava, T. Giraldi, L. Lassiani, and C. Nisi.** Università di Trieste, Italy

BRIEF REPORTS

1757 Phase II Evaluation of 4'-Epi-doxorubicin in Patients With Advanced Colorectal Carcinoma. **R. Michaelson, N. Kemeny, and C. Young.** Memorial Sloan-Kettering Cancer Center, New York, New York

1759 Phase I Study of 13-*cis*-Retinoic Acid Toxicity. **P. R. Band, J. G. Besner, R. Leclaire, C. Girard, G. Diorio, M. Deschamps, M. Gélinas, and D. Larochelle.** Institut du Cancer de Montréal, Quebec, Canada

1763 High-Dose Medroxyprogesterone Acetate in Advanced Male Breast Cancer. **F. Pannuti, A. Martoni, L. Busutti, M. Gramellini, and E. Piana.** S. Orsola-M. Malpighi Hospital, Bologna, Italy

1767 Persistent Hypomagnesemia Following Cisplatin Chemotherapy for Testicular Cancer. **R. L. Schilsky, A. Barlock, and R. F. Ozols.** National Cancer Institute, Bethesda, Maryland

CLINICAL TRIALS SUMMARIES

1771 Tamoxifen in the Treatment of Metastatic Malignant Melanoma. **J. Wagstaff, N. Thatcher, E. Rankin, and D. Crowther.** University of Manchester and Christie Hospital and Holt Radium Institute, England

1773 Phase II Study of Medroxyprogesterone Acetate With Tamoxifen in Advanced Renal Cell Cancer. **J. Z. Fuks, J. Aisner, D. A. Van Echo, and P. H. Wiernik.** University of Maryland Cancer Center, Baltimore

1775 Phase II Clinical Study of Yoshi 864 in Epithelial Ovarian Carcinoma: A Gynecologic Oncology Group Study. **M. Slavik, H. Muss, J. A. Blessing, and G. Delgado.** Gynecologic Oncology Group

1779 Phase II Trial of Mitoxantrone in Previously Untreated Patients With Colorectal Adenocarcinoma: A Southwest Oncology Group Study. **J. D. Cowan, D. D. Von Hoff, B. McDonald, R. W. Talley, J. D. McCracken, and T. Chen.** Southwest Oncology Group

1781 Yoshi 864 Plus Medroxyprogesterone Acetate in Adenocarcinoma of the Kidney: A Southwest Oncology Group Study. **S. J. Altman, R. L. Stephens, and J. D. Bonnet.** Southwest Oncology Group

1783 Failure of 5-Day Vinblastine Infusion in the Treatment of Patients With Advanced Refractory Breast Cancer. **I. Tannock, C. Erlichman, D. Perrault, I. Quirt, and M. King.** Princess Margaret Hospital and Ontario Cancer Institute, Toronto, Canada

LETTERS

1785 Fever Due to 6-Mercaptopurine. **R. Herrmann, C. Manegold, and D. Fritze.** Medizinische Universitätsklinik, Heidelberg, West Germany

1785 Aminoglutethimide-Induced Thrombocytopenia. **B. Ardman and R. A. Rudders.** New England Medical Center Hospital, Boston, Massachusetts

1787 ANNOUNCEMENTS

Chemotherapy for Osteogenic Sarcoma: An Investigative Method, Not a Recipe[1]

Gerald Rosen* and Anita Nirenberg[2]

The controversy over the role of chemotherapy for the treatment of osteogenic sarcoma arises because osteogenic sarcoma has been in the past and always will be a difficult and resistant tumor to treat with chemotherapy. In the majority of instances, the various protocols that have recently proliferated for the treatment of osteogenic sarcoma have been culled from the literature and put together based on theoretic considerations. Whereas this may suffice for the treatment of leukemias and other chemotherapy-sensitive diseases, chemotherapy for osteogenic sarcoma was hard to develop and is rather unique. Unfortunately, dose schedules must be precise with little variation, since experience has shown that the treatment of metastatic osteogenic sarcoma with high-dose methotrexate and leucovorin rescue can be successful only if it is administered in exactly the way that experience has dictated objective responses will occur.

The major breakthrough in the chemotherapy for osteogenic sarcoma is attributed to the work of Jaffe and Paed (1) in 1972 when they showed that massive doses of methotrexate with leucovorin rescue could cause metastatic osteogenic sarcoma to regress. The usual dose initially found to be effective was about 300 mg/kg. Kim and Djerassi (2) suggested that metastatic osteogenic sarcoma might respond to this unique treatment and that there is a threshold dose of methotrexate above which responses occur and below which there is absolutely no response. The use of many other chemotherapeutic agents in other tumors has shown us that even with suboptimal doses there is some partial response to treatment, and more complete responses may be obtained at higher doses. However, the majority of other agents have dose-limiting toxicity. The unique aspect of high-dose methotrexate with leucovorin rescue is that while even small, suboptimal doses such as 50, 100, and 200 mg/kg are potentially as lethally toxic as 300 or 400 mg/kg, it may only be the latter doses that can affect this disease in younger children.

This observation by Kim and Djerassi has been confirmed by our experience at the Memorial Sloan-Kettering Cancer Center, where we have given high-dose methotrexate and leucovorin rescue to > 100 patients with evaluable metastatic disease and > 200 patients with evaluable primary tumors. Experience in the direct observation of patients with evaluable disease who received this treatment has allowed us to determine the optimal dose of high-dose methotrexate for each patient. In addition, extensive pharmacokinetic studies of patients receiving high-dose methotrexate have allowed us to define predictive indicators of impending toxic effects and the medical intervention necessary to prevent severe or even lethal toxic effects (3,4). We have found that it is not enough to ensure the safety of high-dose methotrexate treatment; it is equally important to ensure the effectiveness of treatment. Otherwise, patients may become just as dead from metastatic disease as they would be from severe, lethal toxic effects.

We have reported a 40% complete response rate of osteogenic sarcoma to single-agent, high-dose methotrexate with leucovorin rescue. The overall partial and complete response rate was 68% (5). The doses and scheduling used for these studies were arrived at during 5 years of experience in treating > 200 patients with evaluable disease, with approximately 5000 high-dose methotrexate treatments.

The effective dose of methotrexate was 8 g/m^2 in fully grown adolescents and 12 g/m^2 in younger children who had not reached their adult stature. In addition, pharmacokinetic data have shown that 24-hour methotrexate levels in the range of 10^{-5} M seem to correlate with effectiveness of treatment and that 72-hour serum methotrexate levels > 10^{-7} M can be potentially toxic if leucovorin is not continued until the serum methotrexate level falls below 10^{-7} M. We accomplish the repetition of these safe and effective pharmacokinetics by performing outpatient treatment. We have found that the patient and family are much more reliable in recording and obtaining the correct urine output than are hospital staff. The alkalinization of the urine for the first 24 hours has also prevented renal toxicity, which can lead to prolonged elevated methotrexate levels; the abrogation of the effect of methotrexate on the tumor, due to the need for elevated and prolonged leucovorin administration; and the inability to resume treatment on time. When the patient is sent home following high-dose methotrexate treatment, he and/or his parent checks

[1]Received Mar 22, 1982; accepted Apr 8, 1982.

[2]Departments of Pediatrics (*G. Rosen* and *A. Nirenberg*) and Medicine (*G. Rosen*), Memorial Sloan-Kettering Cancer Center, New York, NY.

**Reprint requests to:* Gerald Rosen, MD, Department of Pediatrics, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021.

the urine at every voiding, and if the pH is not ⩾ 7.0, additional oral sodium bicarbonate is administered. This is difficult to do with inpatient treatment, since busy nurses in the evening and night cannot be expected to immediately check the pH for every patient who voids and immediately administer bicarbonate. Frequently, an acid urine from an inpatient may not come to the nurse's attention for 1–2 hours, and it may be another half hour before the patient receives bicarbonate. This delay alone can be responsible for renal damage and potential severe toxic effects.

To produce the pharmacokinetics of methotrexate elimination that we feel is both safe and effective, patients are instructed, for the first day following high-dose methotrexate, to have a 24-hour urine output of 1500–1600 ml/m^2 of alkaline urine (fig 1). For the next 2 successive days they are instructed to have a urine output > 2000 ml/m^2. In most outpatients who have received extensive training, the urine output is within 100–200 ml of the recommended guidelines.

In these circumstances, we have found high-dose methotrexate with leucovorin rescue to be extremely

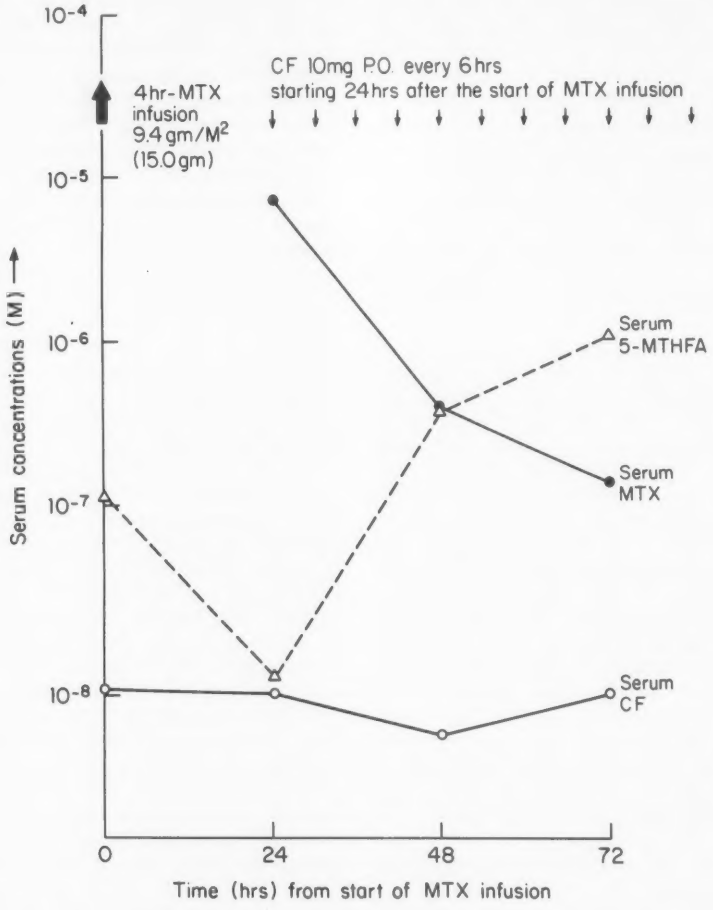


FIGURE 1.—Typical pharmacokinetic profile of serum methotrexate (MTX) and serum 5-methyltetrahydrofolate (5-MTHFA) levels following 15 g of high-dose MTX and 10 mg of leucovorin rescue (citrovorum factor, CF) orally every 6 hrs starting 24 hrs after the start of MTX infusion. The serum MTX level is about 1×10^{-5} M at 24 hrs and about 1×10^{-7} M at 72 hrs. Since CF is converted to 5-MTHFA as it is absorbed in the gastrointestinal tract, CF cannot be measured accurately in the serum. When the oral dosage of CF is 10 mg every 6 hrs, MTX and 5-MTHFA become equimolar in the serum prior to 48 hrs. With these pharmacokinetics, treatment is usually effective and produces no toxicity (*see* fig 2).

effective in the treatment of osteogenic sarcoma (fig 2). Furthermore, we have frequently noted that when hospitalization is required for medical conditions that would not permit the outpatient administration of high-dose methotrexate, a higher percentage of patients fail to respond to therapy and there is a higher incidence of toxic effects.

We have extensively reported on the value of high-dose methotrexate with leucovorin rescue in the treatment of osteogenic sarcoma. Yet no single investigative protocol at another cancer center calls for the use of high-dose methotrexate as we administer it in the outpatient department to reproduce the pharmacokinetics that we have demonstrated to be both safe and effective (figs 1 and 2). The majority of protocols using high-dose methotrexate with leucovorin rescue not only require hospitalization of the patient but specifically call for increased prehydration with iv fluid prior to and following methotrexate infusion. This is directly contradictory to our experience in the effectiveness of high-dose methotrexate in the treatment of osteogenic sarcoma. Giving the treatment as we have described requires 3 days of extensive followup and diligent attention to daily serum methotrexate levels, urine output, and physical condition. If investigators are not willing to wait (sometimes) until 7:00 PM on Friday evening for the biochemistry laboratory to report that day's 72-hour methotrexate levels and to call patients with levels > 10^{-7} M instructing them to continue taking leucovorin, there will be excessive toxic effects with the use of high-dose methotrexate in the outpatient setting. Because of this requirement, we have administered high-dose methotrexate only on Mondays and Tuesdays so a 3-day followup for the serum methotrexate levels could be done on a daily basis to ensure the safety of treatment.

Our first experience in the adjuvant treatment of 54 patients utilized high-dose methotrexate at the dose of 200 mg/kg, doxorubicin at a dose of 90 mg/m^2, and cyclophosphamide at a dose of 40 mg/kg (1200 mg/m^2). The method of administration for this protocol (T-4) is very similar to that for many protocols used in recent years. The overall continuous disease-free survival of patients who were treated with this protocol was only 50%, again comparable to the results being obtained today with other investigative protocols (6,7). The majority of patients treated had not achieved their full adult stature, and we found retrospectively that they had not received effective doses of high-dose methotrexate; that was the area in need of improvement.

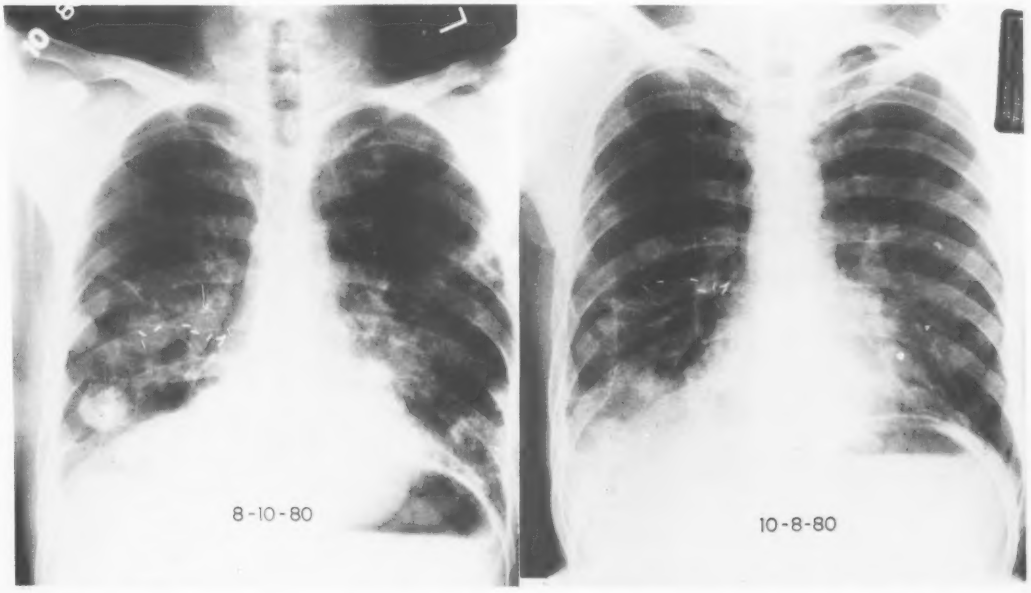


FIGURE 2.—Serial X-rays of a patient who had progressive pulmonary disease while receiving high-dose MTX (15 g) with excessive iv fluid hydration. After referral to Sloan-Kettering he was re-treated at the same dose without excessive fluid hydration; he had a partial response, with a decrease in the size of the large retrocardiac mass as well as other pulmonary metastases.

Further treatment of patients who relapsed with higher doses of methotrexate (12 g/m^2) frequently caused regression of their pulmonary metastases (fig 3).

It was through the use of chemotherapy prior to surgery for osteogenic sarcoma, which was given to make time for construction of an endoprosthesis for limb-salvage surgery, that we were able to observe regression in some primary osteogenic sarcomas when the dose of methotrexate was escalated by 2–4-g increments. The recommendation for the dose of 12 g/m^2 for patients who are not fully grown was made because of that early experience. The observations were also made that no patient responded to a < 12-g dose of high-dose methotrexate and that the escalation to > 20-g doses of high-dose methotrexate seldom yielded any meaningful clinical responses when patients had failed to respond to doses of 16 and 18 g.

These observations and a new dose schedule were incorporated into our T-7 chemotherapy protocol in 1976 (8). In addition, the combination of bleomycin, cyclophosphamide, and dactinomycin (BCD), which had been shown to be effective in the treatment of metastatic osteogenic sarcoma, also replaced the single agent cyclophosphamide. BCD chemotherapy has not been used in any other protocol to date, although we have found it extremely effective, yielding a response rate of approximately 60% (higher than that produced with doxorubicin as a single agent) and with less toxicity than is observed following the use of doxorubicin at the recommended dose of 90 mg/m^2/course (9). We had achieved 82% continuous disease-free survival for all patients treated with the T-7 chemotherapy protocol. All patients treated with that protocol have now been followed for 40–66 months (median, 52) from the start of treatment. The 82% represents continuous disease-free survival and does not include patients who relapsed and were subsequently salvaged with thoracotomy and continued chemotherapy.

Through the use of preoperative chemotherapy and the evaluation of its effect on the primary tumor, we had determined that all patients having a histologically confirmed complete or near-complete (> 90%) response of the primary tumor following the administration of preoperative chemotherapy became continuous dis-

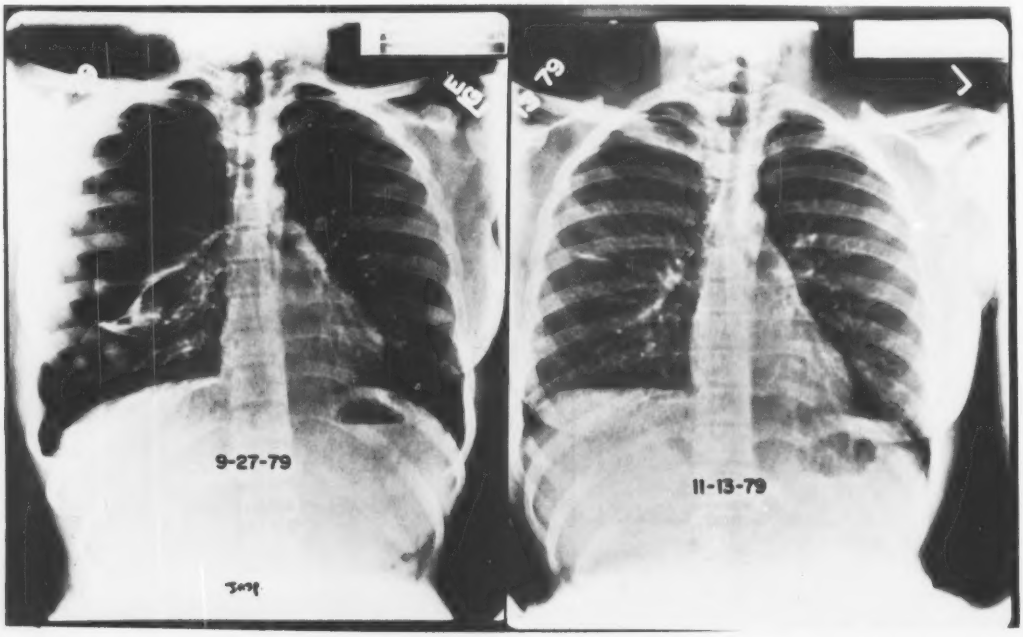


FIGURE 3.—This 13-yr-old girl developed bilateral pulmonary metastases while being treated with a Southwest Oncology Group adjuvant chemotherapy protocol consisting of 3 doses of high-dose MTX (4, 8, and 10 g) interspersed with 2 courses of doxorubicin during 3 mos. She was referred to our clinic for treatment. Note complete disappearance of pulmonary metastases following 4 weekly doses of MTX (14 g; 12 g/m^2).

ease-free survivors, with the exception of one patient who relapsed with a local recurrence after inadequate resection of the primary tumor. All of the relapses occurred in patients whose primary tumor did not respond to preoperative chemotherapy following histologic evaluation of the resected primary tumor (6,8,10).

In 1978, a new protocol (T-10) called for the identification of these poor responders to high-dose methotrexate with leucovorin rescue and other preoperative chemotherapy and the addition of cisplatin to their adjuvant chemotherapy protocol following surgery after high-dose methotrexate was discontinued. This therapeutic maneuver proved to be highly significant in terms of increasing the continuous disease-free survival of patients who did not respond well to chemotherapy prior to surgery. It further raised the continuous disease-free survival of the entire group of (now) 79 patients to > 90% (6–40 months' followup; median, 24) (10).

The majority of patients failing to have a good response to high-dose methotrexate chemotherapy prior to surgery had partial responses; therefore, the addition of cisplatin and continued doxorubicin and BCD following surgery in this group of patients was therapy in addition to the preoperative high-dose methotrexate, which did cause approximately 50% tumor regression in the majority of patients. High-dose methotrexate is probably still necessary in that group of patients since there is some response to it. Further information has shown that the use of cisplatin and doxorubicin alone without prior high-dose methotrexate has led again only to a 52% disease-free survival rate (L. Ettinger, personal communication).

It has recently been claimed that osteogenic sarcoma is becoming a more benign disease (11,12). That has not been our experience (13) nor the experience of other large groups (14,15) studying the natural history of osteogenic sarcoma. However, even if the disease-free survival is between 30% and 40% with surgery alone, it is still far below that which can be achieved with optimal chemotherapy (10). No one would have second thoughts about using adjuvant chemotherapy in diseases that have a 50% cure rate for surgery alone. A one out of two chance of dying of disease in 5 years is unacceptable; patients with tumors such as primary malignant fibrous histiocytoma of soft tissue, with a cure rate following surgery alone that might be as high as 50%, are offered adjuvant chemotherapy, and this is generally accepted by the oncology community as being proper treatment. In addition, the higher cure rate following surgery alone for osteogenic sarcoma is probably related to the earlier diagnosis of small pulmonary metastases by computerized tomographic scan, and those patients are eliminated from the study group. It is precisely those patients who need effective chemotherapy, or the survival rate for that subset of patients will be zero following surgery alone.

Why, then, is there controversy over the treatment of osteogenic sarcoma even if the cure rate can be as high as 40% with surgery alone? The reason is that many investigators have been frustrated in their attempts to successfully treat osteogenic sarcoma and have concluded that if they cannot treat it effectively the treatment is of no value, without stopping to think of how the treatment procedure may be made more effective. Safe and effective high-dose methotrexate treatment is not merely the giving of a drug, it is a pharmacokinetic procedure. It is not an art, it is a precisely spelled-out science based on precise data obtained from each patient following each treatment. Based on the data obtained in this biologically varying milieu, the procedure may have to vary.

At the Memorial Sloan-Kettering Cancer Center, Dr. Ralph Marcove was the first surgical oncologist to employ limb-salvage surgery in the treatment of osteogenic sarcoma (6). In the past, people have commented that our results must be so good because our referral pattern reflects the selection of patients with resectable lesions. This could not be further from the truth of the real situation. It is well-known that by the time patients are referred to tertiary treatment centers they usually have tumors that are further advanced than patients who are treated in regional university centers in their home town. In general, our patients have presented with more and more advanced disease over the years, as has been determined by examining the X-rays and bone scans and the extent of the primary lesion in all of our patients. To date, all but two of 198 protocol-treated patients have presented with moderate to large soft tissue extension of the primary tumor.

In our first chemotherapy protocol (T-4 and T-5, referred to as T-4 for the sake of uniformity), 58% of patients underwent limb-salvage procedures and the rest had amputations (6). In our current protocol (T-10), only 48% of patients have been able to have successful resections (10). We have made chemotherapy prior to surgery much more effective in shrinking primary tumors over the past 8 years, and the surgical techniques have improved as well as the skill and daring of the surgical oncologist in performing limb-salvage procedures. However, the lower proportion of patients who were able to have resection over the past 8 years is a primary indication that patients referred to this tertiary cancer treatment center have more advanced lesions than patients had years ago and that they have more advanced disease than that seen at regional medical centers where the initial biopsy has been performed and definitive treatment started.

The surgical cure rates recently claimed for osteogenic sarcoma without chemotherapy may be artificially high; to determine the effect of chemotherapy on osteogenic sarcoma it is necessary to define subsets of patients with similar prognoses. The most significant

prognostic variable that we have identified to date in the survival of patients with osteogenic sarcoma is the location of the primary lesion. If all of the extremity lesions are considered, it can be seen and is well accepted that patients with primary femur lesions have a worse prognosis than patients with primary lesions of the humerus and tibia. Thus, if a series is weighted with patients who have tibia or humerus lesions, there will be a higher cure rate following surgery alone. In addition, it has been claimed that osteogenic sarcoma patients > 21 years old have a better prognosis. That has not been our experience, but for the sake of uniformity, a specific age group and perhaps a specific site should be designated when disease-free survival rates for osteogenic sarcoma are reported.

During the past 8 years we have treated 185 patients with primary osteogenic sarcoma of the extremity in the age group ≤ 21 years. Ninety-six (> 50%) of the patients had lesions of the femur. In regard to patients with femur lesions alone (fig 4), the continuous disease-free survival of the first 32 patients treated with our T-4 protocol is not much different from that being obtained with various treatment protocols throughout the country at this time (38%) (17–21). However, with refinements in the administration of high-dose methotrexate, in particular the dose of 12 g/m^2 for younger adolescents and children, the continuous disease-free survival rate for patients with femur lesions rose to 65% with the T-7 chemotherapy protocol. With the addition of cisplatin to the treatment of patients not having a complete response to chemotherapy with high-dose methotrexate prior to surgery, the continuous disease-free survival rate for patients with femur lesions rose to 88% with the T-10 chemotherapy protocol. These data include as failures 11 patients (five receiving the T-4 chemotherapy protocol, four receiving the T-7 chemotherapy protocol, and two receiving the T-10 chemotherapy protocol) who failed treatment due to local recurrences in the extremity following what was, retrospectively, inadequate limb-salvage surgery. If one excluded those surgical failures, the continuous disease-free survival rate for each respective protocol would be even higher.

There are those who claim that > 70% of patients can be free of disease at a median time of approximately 2 years with surgery alone, thoracotomy to remove pulmonary metastases, and then delayed chemotherapy (22). In figure 5, the overall survival (not continuous disease-free survival) of our patients is plotted for each of the various protocols; 70% of the patients with femur lesions who received our old T-4 chemotherapy protocol (which we do not consider good chemotherapy at the present time) were surviving at 2 years. Practically all of them were disease-free at that time, since when patients relapsed they had pulmonary metastases removed at thoracotomy; however, they were not continuously free of disease, and many relapsed and died during the next 5 years. These data are as good as, if not better than, any data about surgical treatment for osteogenic sarcoma, since they represent only patients with primary femur lesions, who have a worse prognosis than patients with primary sites in other extremity bones. However, the survival rate of these patients gradually decreased to 37.5% at 7 years, which is not significantly better than the continuous disease-free survival rate of the patients receiving the T-4 chemotherapy protocol (compare fig 5 with fig 4).

We have found that through the use of multiple thoracotomies and continued chemotherapy, it is possible to extend the survival of patients with metastatic disease to between 5 and 8 years from the onset of pulmonary metastases; however, most patients will eventually succumb to recurrent pulmonary metastasis or other distant metastases. To consider the results of surgery alone (including thoracotomy), with chemotherapy added only after pulmonary metastases are resected, the 5- and 6-year disease-free survival rates must be examined. We have found that the continuous disease-free survival rate is the closest correlation to the eventual 6- and 7-year survival rates.

In regard to osteogenic sarcoma in sites other than the femur, figures 6 and 7 show that patients with tibia lesions and humerus lesions seem to have a good prognosis regardless of the chemotherapy protocol used; however, the number of patients treated with the T-4 chemotherapy protocol is too small to say that there are significant differences between the results in T-4-treated patients and the results in patients treated with what we now consider much improved chemotherapy (T-7 and T-10). Nevertheless, the overall continuous disease-free survival rates for both groups of patients are 87% and 88%, respectively, with a 94% continuous disease-free survival rate for patients with tibia and humerus lesions treated with the current T-10 chemotherapy protocol.

One patient treated with the T-4 chemotherapy protocol had a late relapse (after 6 years). He was treated with en bloc resection of a primary osteogenic sarcoma of the tibia, and although not yet proven, it is suspected that a local recurrence may have been the reason for his late failure.

The 185 patients reported above represent all of the patients with primary osteogenic sarcoma of an extremity referred to our service during the past 8 years, except:

(*a*) five patients who presented with simultaneous bone metastases,
(*b*) 27 patients who presented with simultaneous pulmonary metastases,
(*c*) three patients who presented with small cell histology (treated on the Ewing's sarcoma protocol),
(*d*) 22 patients who presented with primary osteo-

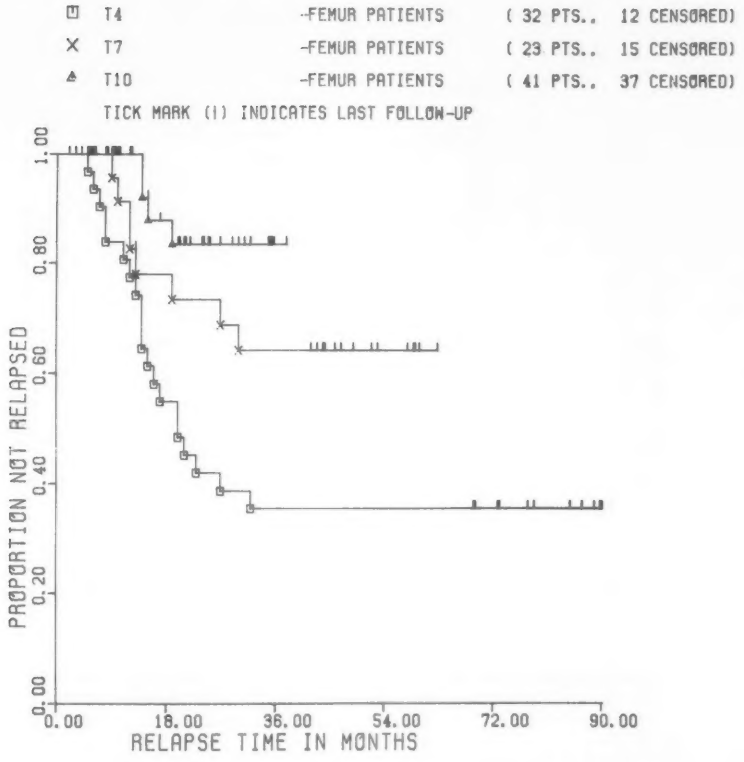


FIGURE 4.—Kaplan-Meier (16) plots of the continuous disease-free survival (DFS) of 96 patients with primary osteogenic sarcoma of the femur, treated on 3 successive protocols over a period of 8 yrs. Censored = No. of patients continuously free of disease. Included with treatment failures are patients with local recurrences following resection (T-4, 5 patients; T-7, 4 patients; and T-10, 2 patients). Overall continuous DFS is projected at 62.5%. Use of 12 g/m^2 of MTX for young children and addition of BCD account for the statistically significant difference in continuous DFS between T-4 and T-7 protocols. Addition of cisplatin for patients not responding to high-dose MTX accounts for the increase in continuous DFS from 65% (T-7) to the projected 3-yr DFS of 82.5% (± 7.0%) with the T-10 protocol.

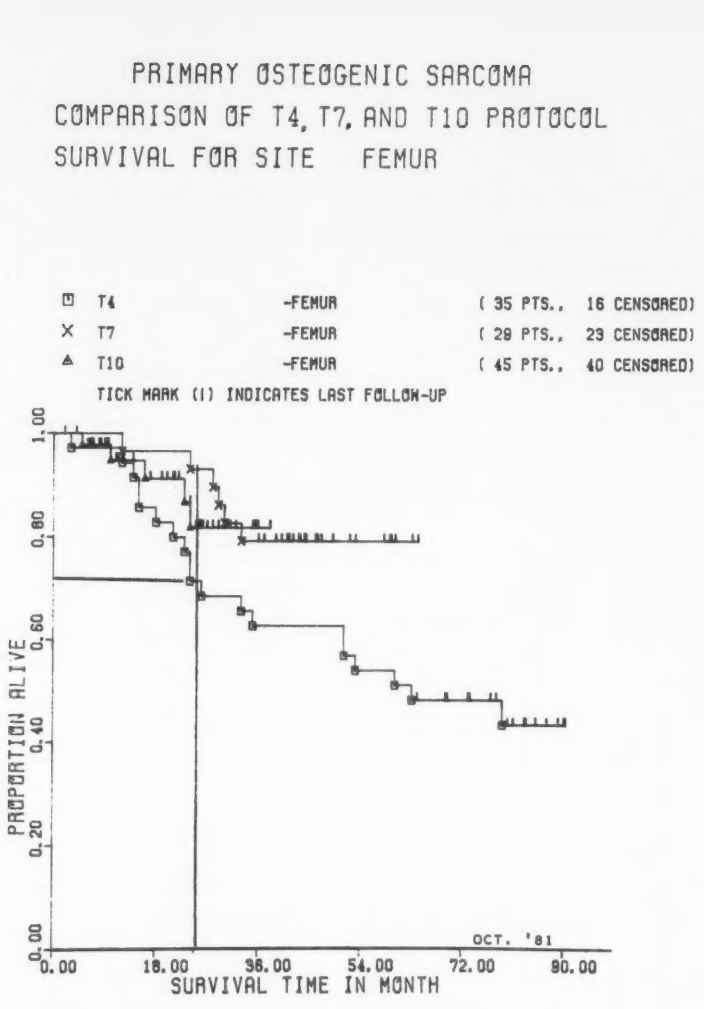


FIGURE 5.—Kaplan-Meier plots of overall survival of patients with primary osteogenic sarcoma of the femur treated with 3 different chemotherapy protocols. The 7-yr survival of the T-4 group is not significantly different from the continuous DFS rate noted in fig 4.

genic sarcoma of an extremity > 21 years old, and

(*e*) three patients who refused to have their initial four high-dose methotrexate treatments at this center and returned to their referring center for treatment.

The requirement that patients receive their initial high-dose methotrexate treatments at this center was included in the eligibility criteria since we believed it was our responsibility to adequately train patients as to what medical intervention may be necessary so that the high-dose methotrexate treatment could be safely and effectively administered in the outpatient department. Because we insisted on this minimal criterion, we have had no patient deaths due to high-dose methotrexate treatment in the past 5 years, representing > 5000 high-dose methotrexate treatments.

It should be noted that in addition to the above exclusions, three patients presenting with low-grade histology were excluded from treatment. On the other hand, three patients whose initial biopsies appeared to show low-grade osteogenic sarcoma later received adjuvant

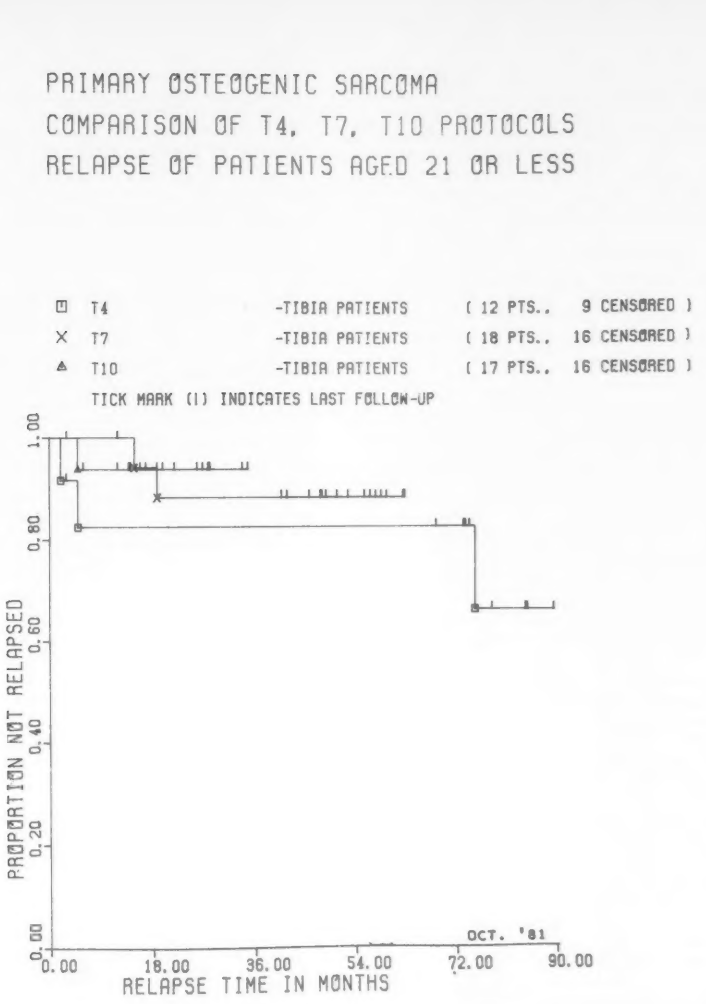


FIGURE 6.—Kaplan-Meier plots of the continuous DFS of patients with primary osteogenic sarcoma of the tibia. Because of the small number of patients treated with the T-4 protocol and the very high continuous DFS rates, there is no statistically significant difference among patients treated with the 3 different protocols.

chemotherapy when high-grade, fully malignant, osteogenic sarcoma was found in the resected or amputated specimen. All 185 patients who were treated with the chemotherapy protocol had osteogenic sarcoma of fully malignant histology (grades III–IV).

From the data presented above, it should be perfectly clear that chemotherapy is effective in increasing the cure rate for osteogenic sarcoma, a malignant neoplasm that in the past has been difficult to treat with chemotherapy but has recently been sensitive to the precisely defined modalities of treatment discussed above. The highest chance for cure in a patient with a malignant neoplasm is achieved through the use of adjuvant chemotherapy prior to the time the patient relapses (23). In addition, the selection of high-risk patients on the basis of their poor histologic response to chemotherapy prior to surgery allows us to institute alternative chemotherapy prior to the patient relapsing, again, at the time when the chance of increasing the cure rate is optimal. This has allowed a further increment in the cure rate for osteogenic sarcoma in our current T-10 chemotherapy study (10).

People have argued that the chemotherapy for osteogenic sarcoma is extremely expensive. This is based

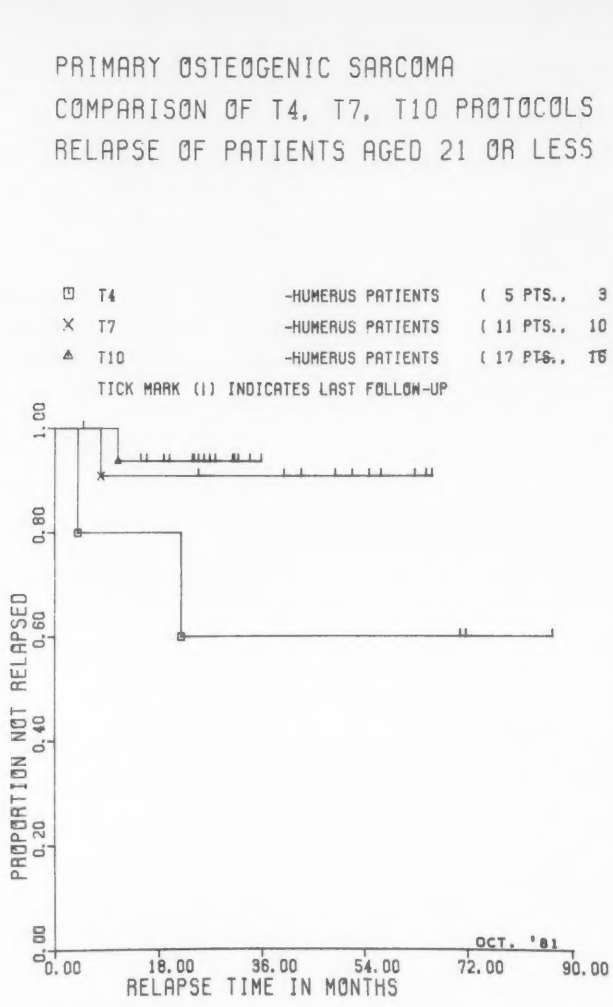


FIGURE 7.—Kaplan-Meier plots of continuous DFS of patients with primary osteogenic sarcoma of the humerus. Because of the small number of patients treated with the T-4 protocol and the very high continuous DFS rates, there is no statistically significant difference among patients treated with the 3 different protocols.

on the price of the 50-mg vial of methotrexate multiplied by 300 to arrive at the expected price of a 15-g dose of methotrexate. This price is artificially high and unwarranted. The actual cost of methotrexate is probably about $10/g, the price that the National Institutes of Health, Division of Cancer Treatment, pays for the high-dose formulation of methotrexate. With wider acceptance of the treatment and the definition of its role in other diseases, the wider demand for methotrexate would probably lower the price that is currently being charged by commercial sources. The current cost to the National Institutes of Health per treatment is ≤ $300, about the cost of a dose of cisplatin (120 mg/m^2). In addition, the cost for a patient bed-day at our hospital is > $450.00. The use of the outpatient department to administer treatment to our patients with osteogenic sarcoma has in effect made the actual cost of their treatment much less than that for patients with other malignancies requiring hospitalization.

Finally, effective chemotherapy for osteogenic sarcoma is difficult to perform and probably should not be performed by those inexperienced in the use of high-

dose methotrexate with leucovorin rescue or by those physicians at institutions where fewer than ten patients per year will be treated. It would require at least this many patients to justify the work involved in monitoring patients following treatment and to give the responsible investigator sufficient experience to safely and effectively treat these patients. In view of the national incidence of osteogenic sarcoma and the number of designated cancer centers, the above consideration would limit the treatment of osteogenic sarcoma to only the larger designated cancer treatment centers. As can be seen from the data presented above, we treated > 50 patients and evaluated the effect of treatment prior to understanding the safe and effective use of high-dose methotrexate. It is unwise for oncologists who will treat fewer than ten patients per year to be involved in the treatment of osteogenic sarcoma. The majority will use protocols that they have little experience with and will have to "process" the patient through the protocol flow sheet. Although the recipe may be the same, the results can be expected to be different.

REFERENCES

1. JAFFE N, and PAED D. Recent advances in the chemotherapy of metastatic osteogenic sarcoma. Cancer 30:1627–1631, 1972.
3. MEHTA BM, JUERGENS H, ALLEN JC, ET AL. Distribution of methotrexate, citrovorum factor, and 5-methyltetrahydrofolate after high-dose methotrexate-leucovorin rescue in osteogenic sarcoma. *In* Current Chemotherapy and Infectious Disease. Proceedings of the 11th International Congress of Chemotherapy and the 19th Interscience Conference on Antimicrobial Agents and Chemotherapy. Washington, DC, The American Society for Microbiology, vol 2, 1980, p 1635.
4. NIRENBERG A, MOSENDE C, MEHTA BM, ET AL. High-dose methotrexate with citrovorum factor rescue: predictive value of serum methotrexate concentrations and corrective measures to avert toxicity. Cancer Treat Rep 61:779–783, 1977.
   otrexate with citrovorum factor resuce: predictive value of serum methotrerate concentrations and corrective measures to avert toxicity. Cancer Treat Rep 61:779–783, 1977.
5. ROSEN G, NIRENBERG A, JUERGENS H, ET AL. Response of primary osteogenic sarcoma to single agent therapy with high-dose methotrexate with citrovorum factor rescue. *In* Current Chemotherapy and Infectious Disease. Proceedings of the 11th International Congress of Chemotherapy and the 19th Interscience Conference on Antimicrobial Agents and Chemotherapy. Washington, DC, The American Society for Microbiology, vol 2, 1980, p 1633.
6. ROSEN G, MURPHY ML, HUVOS AG, ET AL. Chemotherapy, en bloc resection and prosthetic bone replacement in the treatment of osteogenic sarcoma. Cancer 37:1–11, 1976.
7. PRATT CB. Chemotherapy of osteosarcoma. An overview. *In* Therapeutic Progress in Ovarian Cancer, Testicular Cancer and the Sarcomas. The Hague, The Netherlands, Martinus Nijhoff Publishers, 1980, p 329.
8. ROSEN G, MARCOVE RC, CAPARROS B, ET AL. Primary osteogenic sarcoma. The rationale for preoperative chemotherapy and delayed surgery. Cancer 43:2163–2177, 1979.
9. MOSENDE C, GUTTIEREZ M, CAPARROS B, ET AL. Combination chemotherapy with bleomycin, cyclophosphamide and dactinomycin for the treatment of osteogenic sarcoma. Cancer 40:2779–2786, 1977.
10. ROSEN G, CAPARROS B, HUVOS AC, ET AL. Preoperative chemotherapy for osteogenic sarcoma: selection of postoperative adjuvant chemotherapy based upon the response of the primary tumor to preoperative chemotherapy. Cancer 49:1221–1230, 1982.
11. TAYLOR WF, IVINS JC, DAHLIN DC, ET AL. Trends and variability in survival from osteosarcoma. Mayo Clin Proc 53:695–700, 1978.
12. Osteosarcoma: advances in treatment or changing natural history? (Editorial). Lancet 2:82–83, 1978.
13. MIKE V, and MARCOVE RC. Osteogenic sarcoma under the age of 21: experience at Memorial Sloan-Kettering Cancer Center. Prog Cancer Res Ther 6:283–292, 1978.
14. Cancer Registry of Norway. Survival of Cancer Patients—Cases Diagnosed in Norway 1968–1975. Oslo, Norway, The Cancer Registry of Norway, 1980.
15. HARVEL S, and SOLHEIM Ø. The prognosis in osteosarcoma: Norwegian national data. Cancer 48:1719-1723, 1981.
16. KAPLAN EL, and MEIER P. Nonparametric estimation from incomplete observations. J Am Stat Assoc 53:457-481, 1958.
17. SUTOW WW, GEHAN EA, DYMENT PA, ET AL. Multidrug adjuvant chemotherapy for osteosarcoma. Interim report of the Southwest Oncology Group studies. Cancer Treat Rep 62:265–270, 1978.
18. CORTES EP, HOLLAND JF, WANG JJ, ET AL. Amputation and adriamycin in primary osteogenic sarcoma. N Engl J Med 291:998–1000, 1974.
19. FOSSATI-BELLANI F, GASPARINI M, and GENNARI L. Adjuvant treatment with adriamycin in primary operable osteosarcoma. Cancer Treat Rep 62:279–281, 1978
20. ETCUBANAS E, and WILBUR JR. Adjuvant chemotherapy for osteogenic sarcoma. Cancer Treat Rep 62:283–287, 1978.
21. PRATT CB, SHANKS EC, HUSTU HO, ET AL. Adjuvant multiple chemotherapy for osteogenic sarcoma of the extremity. Cancer 39:51–57, 1977.
22. ROSENBERG SA, FLYE MW, CONKLE D, ET AL. Treatment of osteogenic sarcoma. II. Aggressive resection of pulmonary metastases. Cancer Treat Rep 63:753–756, 1979.
23. BURCHENAL J. From wild fowl to stalking horses: alchemy in chemotherapy. Cancer 35:1121–1135, 1975.

Is It Ethical Not To Conduct a Prospectively Controlled Trial of Adjuvant Chemotherapy in Osteosarcoma?[1]

Beverly Lange[2,*] and Arthur S. Levine[3]

The medical literature contains only one report of a randomized, prospectively controlled study comparing surgery alone to surgery plus adjuvant chemotherapy in the treatment of osteosarcoma: the Mayo Clinic study (1). The estimated 2-year survival in this study is 74%, and the estimated 2-year disease-free survival is 52%. No statistically significant differences in survival or disease-free survival exist between the group receiving adjuvant chemotherapy and that receiving surgery alone. The sample size is small, 37 patients at the time of report; the followup is short, a median of 2 years in 1981; and the data have not yet been subjected to peer review (the report mentioned is preliminary, having appeared as a letter to the editor). Nonetheless, this randomized controlled study is the most powerful tool available to date to assess the relative benefits of surgery and chemotherapy in the treatment of osteosarcoma. Its results support two impressions abstracted from many earlier historically controlled or uncontrolled studies: *(a)* that a patient with osteosarcoma diagnosed in the past decade has a better chance of surviving for at least 5 years than a patient with the disease diagnosed earlier in this century and *(b)* that it is not necessarily correct to assume that chemotherapy is responsible for the improvement in survival (2–7). The trend toward improved survival and substantive reservations about the role of chemotherapy have led a number of other institutions to consider a prospectively controlled study comparing surgery alone to surgery plus adjuvant chemotherapy in the primary treatment of osteosarcoma. The purpose of this presentation is to justify such a study in 1982.

Except for the current Mayo Clinic study, the evidence for any improvement in overall survival, unrelated to the use of adjuvant chemotherapy, is largely circumstantial. The Southwest Oncology Group (SWOG) began clinical trials of adjuvant therapy in 1963. Although no improvement in continuous relapse-free survival was seen in the periods 1948–1962, 1962–1968, and 1969–1974, 2-year survival improved from 23% to 40% and 41% during the three respective periods (6,8,9). Presumably, better retrieval of patients who developed metastases accounts for the improvement (9). In 1971, Martini et al (10) documented that resection of pulmonary metastases could greatly prolong the survival of, and possibly cure, as many as 27% of patients with metastatic disease, and Rosenberg et al (11) have shown that 61% of patients with pulmonary metastases could be rendered disease-free by surgical resection. The experience of the Mayo Clinic paralleled that of SWOG in that 2-year survival increased from about 20% before 1968 to 50% between 1969 and 1971. In contrast to the SWOG experience, continuous relapse-free survival at the Mayo Clinic improved from 13% to 20% before 1969 to 42% in 1971 and has continued to improve throughout the decade. Again, while improvement in overall survival can be attributed to the surgical removal of metastases, the evidence suggests that other variables are responsible for the apparent improvement in continuous relapse-free survival (2). The Swedish study of leukocyte interferon as an adjuvant in the treatment of osteosarcoma (12) is a case in point: in 1977, continuous disease-free survival in an interferon-treated group was 50% at 2 years, but disease-free survival in a concurrent but nonrandomized control group was 35% rather than $< 20\%$ as anticipated. More recently, a Norwegian national study (13) has shown that overall survival in patients with osteosarcoma improved from 23% prior to 1975 to 47% after 1975 when adjuvant chemotherapy trials began in that country. Although the Norwegian data demonstrate that the introduction of adjuvant chemotherapy coincided with improved survival, the authors comment that "early amputation, intensified clinical followup, and surgery for metastases may have also contributed to overall survival." All of the studies cited and many others suggest that the impact of adjuvant trials may, in part, have been to render this disease treatable in the minds of physicians who directed patients to definitive surgical attention at an earlier point in time than had been the case historically.

The relatively recent and unexpected improvement in both continuous disease-free survival and overall survival, conceivably independent of, or only indirectly related to, adjuvant chemotherapy, contrasts sharply with all previous experience with this tumor. The natural history of osteosarcoma prior to the widespread use of

[1]Received Apr 5, 1982; accepted Apr 8, 1982.

[2]Division of Oncology, The Children's Hospital of Philadelphia, PA.

[3]Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

**Reprint requests to:* Beverly Lange, MD, Oncology Clinic, The Children's Hospital of Philadelphia, 34th and Civic Center Blvd, Philadelphia, PA 19104.

adjuvant chemotherapy has been reviewed extensively (2–7,14–16). Survival rates were consistently poor here and abroad. Among patients with nonmetastatic osteosarcoma treated with amputation or palliative irradiation, 50% developed metastases 6–10 months from diagnosis, 70%–80% had metastases at 12 months, and only 20% survived for 2 years (1–18). Based on the reproducibility of these numbers from institution to institution and from decade to decade, investigators in the late 1960s and early 1970s understandably concluded that historical controls provided a reliable and valid means of measuring the impact of adjuvant therapy on survival. Needless to say, for historical controls to be a useful statistical instrument, the historical group must be precisely comparable to the current study group. The unexpected improvement rate in 2-year survival to ≥ 40% in patients with osteosarcoma has therefore raised the obvious questions: Are historical and contemporary groups really comparable? And if they are not comparable, why not? Has the natural history of osteosarcoma changed? Has tumor histology or other aspects of biology changed? Have diagnostic maneuvers, surgical intervention, supportive management, or the timing of these events changed? What is the hard evidence that any given chemotherapeutic agent or regimen is effective, either in the treatment of overt disease (primary and metastatic) or in the adjuvant setting?

It is difficult to explain a change in prognosis by a change in natural history. Although theoretically the natural history of a tumor might change, there is no evidence that any form of human cancer has actually changed its biologic behavior. The incidence may change (lung cancer in women) or the host may change (Kaposi's sarcoma as seen in the current epidemic among young male homosexuals), but natural history as determined by biologic behavior seems stable. Factors that may be interpreted as alterations in the natural history of a tumor are more likely indirect manifestations of changes in selection and/or management.

Biologic variables in osteosarcoma surely exist, producing known and unknown prognostic factors, but they are not easily demonstrated and have not even been addressed in most adjuvant studies. The one prognostic variable that most investigators have agreed on historically is site; more distal lesions have been associated with an improved prognosis (7). Tumor size appeared to be an important variable before the chemotherapy era: patients with smaller tumors fared better with surgery alone than patients with large tumors (17). Few adjuvant studies using historical controls have compared mean tumor size. However, a National Cancer Institute (NCI) study showed that a reduction in tumor size was, in fact, apparent: the mean product of diameters of tumors on anteroposterior radiographs was 113 cm^2 in historical controls and 65.9 cm^2 in an adjuvant trial conducted after 1975 (18). This difference may represent a trend toward earlier diagnosis. At any rate, it suggests that current and historical groups may not be comparable with regard to prognostic variables.

The duration of symptoms prior to diagnosis has been cited as another variable, but it is a less objective parameter than tumor site or size, and perhaps it is more subject to changes in diagnostic trends. In the past, a relatively long lag time between the onset of symptoms and diagnosis was considered favorable: it suggested a relatively indolent lesion (7,17). Today, a change in attitude from pessimism to optimism may well bring all patients to an earlier definitive diagnosis. If the assumption that earlier diagnosis is responsible for improved survival is correct, a long lag time will not become unfavorable. Thus, retrospective analysis of apparent changes in the duration of symptoms is likely to be unrewarding and confusing.

Use of the number of patients with overt pulmonary metastases at diagnosis as a method to compare current patients receiving adjuvant therapy with historical controls is complicated by similar confounding variables: increased thoroughness in the search for metastatic disease at diagnosis by whole-lung tomography and computerized axial tomography will eliminate many more patients from the localized-disease category than had been true historically. Retrospective analysis (18–21) has shown, not only that tomograms reveal more lesions, but that the number of patients who would have been diagnosed as having metastatic disease only because of the increased sensitivity of computerized tomography (CT) scanning ranges up to 20%. The use of routine radionuclide bone scanning can similarly alter the composition of the population under study. These variables may play a significant role in changing apparent survival rates; they may narrow the window of patients selected for adjuvant treatment.

Some of the early studies of adjuvant chemotherapy or immunotherapy (eg, interferon) included patients with the less malignant variants of osteosarcoma, parosteal sarcoma, periosteal osteosarcoma, "low-grade intraosseous" osteosarcoma, and chondrosarcoma (16,22). Moreover, not all studies have employed a uniform grading system past and present. At the National Institutes of Health, fewer tumors with histologically high grade (III and IV) were diagnosed after 1975, but this variable alone could not account for the improvement in survival (18). The point is well-taken that histology should be considered as a variable prospectively and that biopsy and surgical specimens should be reviewed by a pathologist who is experienced in the interpretation of malignant bone tumors (23). However, when favorable histologic variants are eliminated from analysis by expert pathologists, survival of patients with osteosarcoma in the past remains at 20% and in the present, ≥ 40%.

Recently, more publicity has attended chemotherapy

than surgery in the treatment of osteosarcoma; however, significant changes in surgical techniques have also taken place in the past decade. As noted above, the innovation that has had the greatest impact on overall survival is the resection, and repeated resections where necessary, of pulmonary metastases. Investigators at the Mayo Clinic, Memorial Hospital, NCI, and elsewhere have demonstrated that many patients who develop pulmonary metastases can be rendered disease-free for years by aggressive removal of their lesions (2,8,10,11). Other changes include the greater use of transmedullary amputation and various limb salvaging procedures. The development of limb salvaging procedures may indirectly have influenced the duration of continuous disease-free survival by reducing the anxiety associated with the prospect of amputation, thereby expediting early referrals of patients to cancer centers, ie, setting the clock that times the interval until metastases are detected at an earlier hour.

Other variations in surgical techniques need to be addressed when surgery alone (historically and currently) is compared to surgery plus adjuvant chemotherapy. Surgeons at the Mayo Clinic, which has the best results with surgery alone to date, do not use a proximal tourniquet at biopsy; they evaluate the biopsy material by frozen section and proceed directly with definitive ablative surgery (24). Most institutions do not have pathologists who are experienced in interpretation of malignant bone tumors or surgeons prepared to amputate on the basis of a frozen section. It is possible that no other institutions will be able to reproduce the Mayo Clinic's success with surgery alone unless they too eliminate the delay of days to weeks sometimes occasioned by two-stage operations.

At this point, no single variable has emerged to explain the differences in survival between historical controls and current patients. It may well be that the combination of all of the factors noted above has contributed to both an apparent and real improvement in survival. However, the improvement in survival has coincided with the introduction of organized trials of adjuvant chemotherapy. The selection of agents for use in these trials was based on phase I and II studies (7,25) of chemotherapeutic agents and combinations in patients with measurable disease; these studies have unequivocally demonstrated that some agents are tumoristatic and infrequently tumoricidal in a therapeutic setting. Some early studies made no distinction between stabilization of disease, partial response, mixed responses, and objective versus subjective responses. Moreover, some response data are confounded by intercurrent exposure of the patient to radiotherapy and/or surgery. The list of agents or combinations that have yielded objective tumor regression in more than one or two patients in any series is short, and the number of patients reported is small. The following agents and combinations have demonstrated activity: doxorubicin and cisplatin as single agents; high-dose methotrexate and leucovorin calcium (with or without vincristine); the combination of bleomycin, cyclophosphamide, and dactinomycin (BCD) (26); and in adults, doxorubicin and dacarbazine. Only the efficacy of doxorubicin (27,28) and high-dose methotrexate (29,30) have been confirmed at more than one institution. Although early studies documented response rates of 40%–80%, recent, more conservative estimates suggest that objective tumor regression occurs in 10%–20% (with the highest response, 37%, reported for BCD) (26–31). Moreover, in most studies, complete responses to any drug or combination have been rare, comprising $< 10\%$ of all responsive cases (7). These studies in patients with overt disease provide the only clues as to which of these drugs and combinations might benefit patients when given as adjuvant therapy and also suggest that the impact of adjuvant therapy might be modest at best.

In many adjuvant trials, doses and schedules of the agents employed differ from those used earlier in treating overt disease; they also differ from trial to trial. While the three successive adjuvant trials at the Sidney Farber Cancer Institute have suggested a dose-response effect with high-dose methotrexate (32), the Children's Cancer Study Group (in CCSG study 741) has failed to demonstrate any advantage of high-dose methotrexate over moderate-dose methotrexate (I. Ertel, personal communication). It had been thought that serum levels of methotrexate in the 10^{-4} M range were necessary to penetrate into osteosarcoma (levels not achieved with moderate-dose methotrexate), but the CCSG and others report a survival rate $> 40\%$ in patients treated with lower concentrations. Furthermore, $> 40\%$ of patients treated with adjuvant combinations previously judged as inactive or untested as single agents (ie, vincristine, bleomycin, dactinomycin, low-dose methotrexate, melphalan, and prophylactic pulmonary irradiation) now survive (7). More than 40% of patients treated with noncytotoxic agents of highly questionable effectiveness in this disease also survive; these agents include transfer factor (33), tumor cell vaccine (34), warfarin (Coumadin) (35), and interferon (12). Compared to the widely quoted series of historical controls, virtually all adjuvant agents have yielded statistically significant improvements in survival.

The very diversity of agents and techniques credited with improving disease-free survival in this notoriously resistant tumor led Carter and Friedman (5) to observe that "with more careful case selection combined with earlier data analysis, almost anything can seem to be positive when compared to the earlier data base." It is also curious that in most studies, the combination of active drugs (eg, high-dose methotrexate with or without doxorubicin) did not improve results, unlike the situation with most other responsive tumors. These re-

sults suggest either that no adjuvant treatment is effective or that all are equally effective; ie, no more cells are killed even though more drugs with different intracellular targets (and different mechanisms of resistance) are added. Finally, while the initial life-table analyses in the first adjuvant reports projected relapse-free survival rates > 80% (8,16,36), actual 2-year, relapse-free survival rates now range from 26% to 65% (6,7), not dissimilar to disease-free survival in contemporary control subjects treated with surgery alone or with presumably ineffective therapy.

Thus, > 40% of patients treated with adjuvant agents that are generally accepted as active in overt osteosarcoma (ie, methotrexate, doxorubicin, and cisplatin) now survive, continuously free of disease (7). When the results of various adjuvant regimens are compared, the differences usually fail to reach statistical significance because of wide ranges in confidence limits (the sample size in most trials is small) and because of the relatively narrow range of results. Two exceptions are results in patients who were treated according to the T-7 or T-10 protocol at Memorial Hospital, where continuous relapse-free survival at a median of 2 years is > 90% (37,38), and in patients at the Sidney Farber Cancer Institute who were treated according to the third consecutive adjuvant protocol (32). These exceptions provoke the question of the role of the investigator or institution in influencing outcome. Investigators at these two institutions have been pioneers in the treatment of osteosarcoma; their experience is more extensive, and possibly their commitment to detail is more intense, than in institutions that are trying to reproduce their results. It is not unusual that the institution that has developed a technique remains the most adroit and successful in its application. However, it is also not unusual for second-generation studies to demonstrate that an untreated or conventionally treated control group has better results than anticipated (39).

If we accept as a given that a highly predictable 40%–50% of all osteosarcoma patients treated with a diversity of adjuvant agents and regimens now survive continuously free of disease for ≥ 2 years, there remain the questions of why Rosen et al (37), using an approach that does not differ radically from that of others, report a figure for continuous disease-free survival > 90%. Certainly, extensive experience and commitment to detail may account for some of the difference. However, it is difficult to conceive that increasing the dose of methotrexate to > 8 g/m^2 in young children—the point that Rosen emphasizes—accounts for this apparently striking improvement. Only a minority of the osteosarcoma population is sufficiently young (ie, remote from achieving their full adult stature) that this point is germane, and the results in this limited group should therefore not have great impact on the overall results. Moreover, the CCSG study, as discussed above, fails to reveal any dose-response relationship within the range employed by that group. Alternative explanations for this striking difference can be entertained: Rosen (38) states that the number of patients who are eligible for limb salvage operations at his institution has been falling, ie, patients are now being referred with more advanced lesions, beyond the point of resectibility. If such patients still are free of metastases on referral to Memorial Hospital, as determined by sensitive CT scanning, they must surely have a biologically favorable disease, possibly with a reduced propensity for early metastatic spread.

Because of coincidence in the use of adjuvant chemotherapy and improvement in survival in osteosarcoma and because of the proven efficacy (however slight) of some agents in patients with measurable disease, adjuvant chemotherapy continues to be used in an uncontrolled fashion in most cancer centers throughout the world. However, the apparent and unexpected improvement in continuous disease-free survival since 1970, in a small number of patients treated only surgically, has so confounded the interpretation of results of adjuvant chemotherapy trials that a growing number of investigators, in addition to those at the Mayo Clinic, have come to believe that they are ethically and intellectually obliged to conduct a randomized, prospectively controlled study comparing surgery alone to surgery plus adjuvant chemotherapy. When, in the mid-1970s, reservations about a cause and effect relationship between adjuvant chemotherapy and survival were first raised and when prospectively controlled studies were proposed, such trials were considered to be nihilistic and frankly unethical. The cost, the toxicity, and the psychosocial disruption imposed by adjuvant chemotherapy regimens were thought to be far outweighed by the benefits that then appeared newly available to all patients with osteosarcoma. Furthermore, the tremendous publicity afforded this apparent success made any controlled study difficult to undertake because of the demands for chemotherapy made by referring physicians and patients. Thus, in 1982, after scores of clinical trials, hundreds of abstracts and manuscripts, and extensive agonizing over what constitutes a responsible approach, we still do not know if adjuvant chemotherapy improves continuous disease-free survival or overall survival in most patients with osteosarcoma. It seems evident that osteosarcoma is a relatively unresponsive tumor with respect to the majority experience using currently available chemotherapy and radiotherapy and that much of the enthusiasm for chemotherapy in this disease, whether as an adjuvant to resection of the primary tumor or to metastatectomy, has been based on results that may reflect subtle distortions in case selection, assessment, and prognostic factors, as well as the possibility of observer bias and insufficient followup in such an enthusiastic

climate. Moreover, interpretation of long-term survival (as opposed to cure) is presently complicated by the frequent failure to distinguish patients with continuous disease-free status after one thoracotomy from patients who have had multiple sequential thoracotomies, including very recent ones.

If chemotherapy were innocuous, one might give the results of adjuvant chemotherapy the benefit of the doubt. However, the long-term effects of present regimens have not been completely evaluated. Recently at the NCI, cardiac abnormalities have been demonstrated by gated-ECG radionuclide scanning in 50% of young adults treated with doxorubicin as an adjuvant to surgical resection of soft tissue sarcomas, and congestive heart failure has occurred in 16% of such patients. This is but one possible morbid sequel of the regimens currently used and serves to emphasize the necessity that we be convinced at a statistically significant level of the ultimate risk-to-benefit ratio of these treatments. For this reason, investigators of the Pediatric Oncology Group, the National Cancer Institute, the Sidney Farber Cancer Institute, Stanford University, St. Jude Hospital, M. D. Anderson Hospital, the Children's Hospital of Philadelphia, and other institutions are now beginning a multi-institution, prospectively controlled (surgery alone), randomized clinical trial of a regimen approximating that reported to have yielded the best results to date (the T-10 protocol described by Rosen [38]). This study appears to be long overdue. It is certainly true that a large multi-institution study may not yield as good a result as Rosen's single-institution trials, but analysis of the results and quality of participation by center will address this point.

REFERENCES

1. Edmonson JH, Green SJ, Ivins JC, et al. Methotrexate as adjuvant treatment for primary osteosarcoma. N Engl J Med 303:642–643, 1980.
2. Taylor WF, Ivins C, Dahlin D, et al. Trends and variability in survival in osteosarcoma. Mayo Clin Proc 53:695, 1978.
3. Muggia F, and Louis AC. Five years of adjuvant treatment of osteosarcoma: more questions than answers. Cancer Treat Rep 62:301–306, 1978.
4. Muggia F, Catani R, Lee YJ, et al. Factors responsible for therapeutic success in osteosarcoma. *In* Adjuvant Therapy of Cancer, 2nd ed (Jones S, and Salmon S, eds). New York, Grune and Stratton, 1979.
5. Carter SK, and Friedman M. Osteogenic sarcoma treatment overview and some comments on interpretation of clinical trial data. Cancer Treat Rep 62:199–204, 1978.
6. Carter SK. The dilemma of adjuvant chemotherapy for osteosarcoma. Cancer Clin Trials 3:29–36, 1980.
7. Bode U, and Levine AS. The biology and management of osteosarcoma. *In* Cancer in the Young (Levine AS, ed). New York, Masson Publishing, 1982.
8. Sutow WW, Sullivan MP, Fernbach DJ, et al. Adjuvant chemotherapy in primary treatment of osteogenic sarcoma. Cancer 36:1598–1612, 1975.
9. Gehan EA. Comparative clinical trials with historical controls: a statistician's view. Biomedicine 28:13, 1978.
10. Martini N, Huvos A, Miké V, et al. Multiple pulmonary resections in the treatment of osteogenic sarcoma. Ann Thorac Surg 12:271–280, 1971.
11. Rosenberg SA, Flye MW, and Conkle D, et al. The treatment of osteosarcoma. II. Aggressive resection of pulmonary metastases. Cancer Treat Rep 63:753–762, 1979.
12. Strander H. The interferon system and its possible use in treatment of neoplastic disease. Cancer Immunother 3:35, 1977.
13. Harvei S, and Solheim O. The prognosis in osteosarcoma: Norwegian national data. Cancer 48:1719–1723, 1981.
14. Marcove RC, Mike V, Hajek JV, et al. Osteogenic sarcoma in childhood. NY State J Med 71:855–859, 1971.
15. Dahlin DC, and Coventry MB. Osteosarcoma, a study of 600 cases. J Bone Joint Surg 49A:101–110, 1967.
16. Jaffe N, Frei E, Traggis D, et al. Adjuvant methotrexate and citrovorum factor treatment of osteosarcoma. N Engl J Med 291:994–997, 1974.
17. McKenna RJ, Schwinn CP, Soong KY, et al. Sarcomata of the osteogenic series (osteosarcoma, fibrosarcoma, chondrosarcoma, parosteal osteogenic sarcoma, and sarcoma arising in abnormal bone): an analysis of 552 cases. J Bone Joint Surg 48-A:1–26, 1966.
18. Rosenberg SA, Chabner B, Young R, et al. The treatment of osteosarcoma. I. Effect of adjuvant high-dose methotrexate following amputation. Cancer Treat Rep 63:739–752, 1979.
19. Muhm J, Brown L, and Crow J. Detection of pulmonary nodules by computed tomography. AJR 128:267–270, 1977.
20. Cohen M, Smith W, and Westman R. Efficiency of whole lung tomography in diagnosing metastasis from solid tumors in pediatric patients. AJR 135:865, 1980.
21. Smoger R, Rosenberg HK, Kasa J, et al. The search for pulmonary metastases in pediatric patients: CT vs conventional radiology. Ann Rad, 1982. In press.
22. Proceedings of the International Workshop on Interferon in the Treatment of Cancer. New York, NY, Mar 31–Apr 2, 1975, p 44.
23. Dahlin DC. Problems in interpretation of results of treatment of osteosarcoma. Mayo Clin Proc 56:621–622, 1979.
24. Sim FH, Ivins JC, and Pritchard DJ. Surgical treatment of osteosarcoma at the Mayo Clinic. Cancer Treat Rep 62:205–212, 1978.
25. Proceedings of the Osteosarcoma Study Group Meeting. Cancer Treat Rep 62:187–312, 1978.
26. Mosende C, Gutierrez M, Caparros B, et al. Combination chemotherapy with bleomycin, cyclophosphamide and dactinomycin for the treatment of metastatic osteosarcoma. Cancer 40:2779–2786, 1977.
27. Cortes EP, Holland JF, Wang JJ, et al. Doxorubicin in disseminated osteosarcoma. JAMA 221:1132–1138, 1972.
28. Gottlieb JA, Baker LH, O'Bryan RM, et al. Adriamycin (NSC-123127) used alone and in combination with soft tissue and bony sarcomas. Cancer Chemother Rep Part 3, vol 6(2):271–282, 1975.
29. Jaffe N. Recent advances in the chemotherapy of metastatic osteosarcoma. Cancer 30:1627–1631, 1972.
30. Pratt CB, Howarth C, Ransom JL, et al. High-dose methotrexate used alone and in combination for measurable primary or metastatic osteosarcoma. Cancer Treat Rep 64:11–20, 1980.
31. Lange B, Kramer S, Gregg R, et al. High-dose MTX and Adriamycin for osteosarcoma: The Children's Hospital of Philadelphia Study. Am J Clin Oncol 5:3–10, 1982.
32. Goorin A, Link M, Jaffe N, et al. Adjuvant chemotherapy (chemo) and limb-salvage procedures for osteosarcoma—a seven-year experience. Proc Am Assoc Cancer Res and ASCO 21:472, 1980.
33. Gilchrist GS, Ivins JC, Ritts RE, Jr. et al. Adjuvant therapy for nonmetastatic osteosarcoma: an evaluation of transfer factor vs combination chemotherapy. Cancer Treat Rep 62:289–294, 1978.
34. Marcove RC, Martini N, and Rosen G. The treatment of pulmonary metastases in osteosarcoma. Clin Orthop 65:70, 1975.

35. HOOVER HC, KETCHAM AS, MILLER RC, ET AL. Osteosarcoma: improved survival with anticoagulation and amputation. Cancer 41:2475, 1978.
36. CORTES EP, HOLLAND JF, WANG JJ, ET AL. Amputation and adriamycin in primary osteosarcoma. N Engl J Med 291:998–1000, 1974.
37. ROSEN G, HUVOS AG, NIRENBERG A, ET AL. Osteogenic sarcoma: selection of adjuvant chemotherapy based upon the response of the primary tumor to preoperative chemotherapy. Proc Am Assoc Cancer Res and ASCO 22:429, 1981.
38. ROSEN G, and NIRENBERG A. Chemotherapy for osteogenic sarcoma: an investigative method, not a recipe. Cancer Treat Rep 66:1687–1697, 1982.
39. SCHNEIDERMAN MA. How do you know you have done any better? Cancer 35:64–69, 1975.

The Development of New Cancer Drugs: A Joint Venture in Public Policy by the National Cancer Institute and Food and Drug Administration[1,2]

J. Richard Crout[3,*]

I am delighted and honored to participate in your program this afternoon. It is a mark of Dr. John Ultmann's generosity as president of the American Society of Clinical Oncology, and of his sensitivity to public affairs, that I am here. Last fall we both appeared at a Congressional hearing, where I discussed a series of innovative policy agreements that have been made in recent years between the National Cancer Institute (NCI) and the Food and Drug Administration (FDA) to foster the development of cancer chemotherapeutic agents. I was of course very pleased when he called some time later to ask if I would share his presidential address time at this meeting to report to you on this same topic. I cannot imagine a warmer gesture or a better forum, and I am very grateful to him.

Let me begin by reminding you, in broad terms, how new cancer drugs are developed and distributed in this country. Most proposed new cancer chemotherapeutic agents, even those originally discovered by drug firms, funnel through the NCI evaluation program. Under this program many new molecules are tested for antineoplastic activity in animals, and a few are selected for subsequent toxicologic testing in animals. From this group are selected the compounds for evaluation in phase I and II clinical trials. If a compound appears to be effective in humans on the basis of remission rates for one or more tumor types, it is evaluated more broadly in phase III trials conducted by cooperative groups and individual investigators. If the drug is especially promising for a particular tumor type by comparison with other available therapy, a demand for use of the drug in treatment develops at this point, even though it is not yet marketed. It has long been NCI and FDA policy to permit so-called treatment or compassionate distribution of an investigational drug to cancer patients under these circumstances. Later still in the developmental process, a drug firm will be licensed by the NCI to combine the animal and clinical data on the drug and submit this information to the FDA in an application called a new drug application (NDA). After review and approval of this NDA, the drug is marketed by a manufacturer in the usual manner for all drugs.

The point I would emphasize is that all of the drugs distributed by the NCI are in one stage or another of the investigational process and are investigational under the law. This means that all such distribution must be for research or treatment under an investigational new drug application (IND) submitted to the FDA. Such use thus requires research protocols, written informed consent, institutional review board (IRB) review, and the mandatory reporting of serious adverse reactions; these are all requirements for investigational drugs but not for marketed drugs.

Against this background, let me comment briefly on certain problems that developed between the NCI and the FDA in the mid-1970s. For a variety of reasons that seemed to come together at about the same time, both institutions came to realize that they needed to do a better job of managing the whole process for investigational cancer drugs. Also, tension developed between the institutions over such matters as the quality of applications, late annual reports, and our respective roles. The situation came to a head when the FDA turned down several INDs, some for procedural reasons and some for scientific disagreement over the safety of proposed studies. You are familiar with the outcry against the FDA regulation by some members of the cancer community at that time.

It was in that environment that Dr. Vincent DeVita (then director of the Division of Cancer Treatment, NCI) and I, along with our staffs, began to undertake the joint ventures that are the central theme of this talk.

First, we developed a memorandum of understanding signed by the Director of the National Institutes of Health and the Commissioner of Food and Drugs outlining the respective roles of the two institutions. NCI reaffirmed its policy of following all FDA regulations with respect to investigational new drugs. FDA agreed to accept from NCI a master file detailing its internal procedures and providing general information applicable to all NCI applications, thereby reducing the need for redundant submission of paperwork. FDA also agreed not to turn down INDs from NCI on procedural or paperwork grounds. Instead we would use the master file as the focus for discussion for procedural problems and individual INDs as the focus for discussion of scientific issues on specific drugs. We also agreed as a matter of internal policy to permit NCI to correct deficiencies in

[1]Received May 18, 1982; accepted June 8, 1982.
[2]Delivered at the National Meeting of the American Society of Clinical Oncology, St. Louis, MO, Apr 26, 1982.
[3]Bureau of Drugs, Food and Drug Administration, Rockville, MD.
*Reprint requests to: J. Richard Crout, MD, Office for Medical Applications of Research, National Institutes of Health, Bldg 1, Rm 216, Bethesda, MD 20205.

its INDs without delaying clinical trials unless a deficiency posed a clear safety issue. NCI in turn increased its staff to improve the quality of its INDs. The result of all these changes has been very positive: a smooth IND process for the past several years, with no significant delays in processing INDs submitted by the NCI.

Second, NCI carefully drafted, and FDA carefully reviewed, the master file describing the NCI drug development and distribution system. The result has been a far greater understanding of the other institution by both staff and management of NCI and FDA.

Third, NCI greatly revamped its drug distribution system by classifying its investigational drugs into three categories known as A, B, and C. Group A drugs correspond roughly to those in phase I and early phase II, while group B drugs are those in late phase II and phase III of the investigational process. Group C drugs are those investigational drugs with sufficient evidence of particular value in the treatment of a tumor type that they are made available to oncologists for patients with those types of cancer. The group C category thus provides a legal mechanism for investigational drugs of special promise to be made available for the treatment of patients. Before a drug goes into group C, NCI supplies the available information to FDA and the data are reviewed by our Oncological Drugs Advisory Committee. The committee's reviews are thoughtful and rigorous. They commonly occupy several hours of a meeting, and although uncommon, turndowns on grounds that the evidence is premature have occurred.

Fourth, NCI proposed a modified set of guidelines for toxicologic studies of new cytotoxic drugs prior to their evaluation in humans. The new guidelines eliminate testing in the monkey, decrease the studies conducted in dogs, and increase testing in mice. These new guidelines have proven to be the most controversial of the policy changes adopted in recent years and, to some extent, are still evolving with experience. Nevertheless, they have been reviewed extensively by FDA, NCI, Public Health Service, and several outside advisory groups, and I expect the basic approach in these guidelines to remain unchanged so long as our experience remains favorable. I would emphasize to this audience that those of you who conduct clinical trials of cancer drugs have a critically important role in determining the success of this new toxicologic approach. The primary intent of animal testing under the new guidelines is to estimate a safe initial dose for the beginning of phase I trials. It is not to establish the organ toxicity of the drug, the usual objective in most toxicologic work. The strategy adopted by the guidelines is that, regardless of the findings in animals, potential toxicity to any organ must be presumed for this highly toxic class of drugs and be specifically sought in humans. The burden for identifying and limiting organ toxicity in patients has, of course, always rested with those who study these agents in clinical trials and use them in the care of patients. The strategy followed in the new guidelines makes that burden explicit.

Fifth, representatives of FDA and NCI, at both staff and management levels, began to meet periodically to discuss problems of mutual concern. These meetings have increased understanding and have served to identify problems at an early stage.

I feel confident today that the major problems of the past are solved as a result of the policy steps taken jointly by the FDA and NCI. Several INDs for new chemotherapeutic agents have been received from the NCI and approved in the past year. The future looks bright and busy for new drug development in the cancer area.

However, all controversy has not disappeared, nor has life suddenly become dull at either the FDA or the NCI. There will continue to be occasional scientific disagreements in interpreting the data on individual drugs. The two institutions have different roles and, like authors and editors or athletes and coaches, are inextricably bound in a common enterprise. But the product of that enterprise, extracted though it may be with some Sturm und Drang, is better than either could produce alone.

Because people are human, there will also continue to be differences over the policies applied to new drug development and regulation. For every person who believes that the regulation of cancer drugs is too lax, there is someone else who thinks that the FDA should not regulate such drugs at all. For everyone who feels that the new toxicologic guidelines are too sparse, there is someone else who wants to extend them to other classes of drugs. For those who believe in the particular value of randomized controlled trials, there is someone else to argue the value of historical trials. These controversies are likely to continue indefinitely. If one of them erupts now and then in the press or a Congressional hearing, it is not to be taken as a sign that something is wrong with the regulatory process. Neither does it mean that every voice represents an institution or is announcing new policy. Such eruptions may simply be a sign that intelligence and thoughtful debate are still alive in Washington—something I gather you wonder about from time to time.

I would like to take the opportunity to pose three challenges to your specialty; all are narrowly focused and relate to the development and regulation of cancer drugs. The first challenge concerns the quality of informed consent by patients who participate in cancer trials, particularly phase I studies. While the articles in *The Washington Post* last fall have been widely recognized as inaccurate and unbalanced, there was running through them one seemingly credible theme, namely that at least some patients do not have a full picture of

the potential risks and benefits of phase I studies. The same theme was echoed in the related Congressional hearings. I do not presume to know where righteousness lies on this complex issue. However, I do believe that I can observe an undercurrent of concern in the Washington press and in the Congress over whether all patients in phase I studies are being approached forthrightly and honestly. My observation is that those of you who face the problem of helping patients with difficult choices at the end of their lives are perceived, whether deserved or not, to have both a challenge and an opportunity: a challenge to assure balance in the informed consent process and an opportunity to win greater public trust in the early drug development process.

A second challenge I would pose to you is to maintain a high sensitivity to the potential for discovering new serious adverse reactions to cancer drugs. History tells us that such effects, particularly those that are delayed or cumulative, are discovered almost entirely by astute clinicians who are prepared to see the new and novel. I would urge each of you to be alert to the contribution to drug safety that you can make through perceptive observation.

Finally, I believe we have a common challenge: to improve the regulation of all drugs, including cancer drugs. I recognize that it is popular to think of regulation as something that occurs in Washington apart from the rest of the country. I recognize also that the escalation of regulatory requirements or procedural intricacies is commonly attributed to empire building by bureaucrats. I would not deny that there is some truth in both statements, but I would caution against accepting these popular dicta too easily. The real problem, as I see it, is a phenomenon known as "regulatory creep," which occurs for the same reasons that technologic advance or procedural complexity occur in other fields. Regulatory creep occurs because good people who are intelligent and well-motivated want to do a better job. A toxicologist wants to add a new test because it is technically possible and potentially helpful. A data-processing expert wants to add a new form to make his or her work more efficient. A dean wants to add another committee review in the interest of winning broader consensus in the institution. An investigator wants to add additional laboratory tests to a clinical trial as part of a personal research interest. And so it is that we gradually make the simple complex and the straightforward costly.

The solution is not simply to blame the regulators. The solution is to work together to find the many small ways in which we can each contribute to the reversal of regulatory creep—through greater self-restraint, by building trust, by keeping review procedures to a minimum, and by encouraging innovation and freedom as well as accountability in the conduct of clinical research. The FDA is working hard to revise its regulations governing the investigational drug process, with these principles in mind. While it will be some time before a revised version of these regulations can be published, I believe the agency will ultimately improve the process without compromising the basic essentials of our drug regulatory system. To the extent that you too can limit procedural and technical requirements in committee reviews in your own hospitals and medical schools, we will together make progress in the search for simplicity.

In closing, I thank Dr. Ultmann again for the privilege of joining your program. The combined efforts of our nation's biomedical research program, our drug and medical device industries, and your specialty of medicine are now paying off in the ever-improving survival of many cancer patients. That is an enormous medical achievement. We at the FDA share your pride in accomplishment and your hopes for the future.

Vindesine: An Effective Agent in the Treatment of Non-Small Cell Lung Cancer[1,2]

Becky E. Furnas, Stephen D. Williams, Lawrence H. Einhorn,* and Melody A. Cobleigh[3]

A dose of 3–4 mg/m² of vindesine (DVA) was given by iv push weekly to 33 patients with non-small cell lung cancer. Twenty-nine patients were evaluable for response. All evaluable patients had a performance status > 50% and measurable disease, and received at least two consecutive weekly doses of DVA. Standard response criteria were used. Among 15 patients with squamous cell carcinoma there were four partial responses and one complete response; three of eight patients with adenocarcinoma had partial responses. The overall response rate was 28% (eight responses among 29 patients). Myelosuppression was moderate, but two patients were hospitalized for granulocytopenic infections. The most common dose-limiting side effects were due to neurotoxicity. Four responding patients had treatment stopped because of neurotoxic reactions. DVA appears to be among the most active drugs in non-small cell lung cancer, but improved dose schedules that reduce neurotoxicity would be highly desirable. [Cancer Treat Rep 66:1709–1711, 1982]

Vindesine (DVA) is a semisynthetic vinca alkaloid derived from vinblastine (1). Phase I studies by Dyke and Nelson (1) recommended that DVA be given at a dose of 3–4 mg/m²/week. In early laboratory trials it appeared that neurologic toxicity with DVA would be less than that with previously tested vinca alkaloids (2).

A partial remission in one of four patients with squamous cell lung cancer was first reported by Bodey and Freireich in 1976 (3). Table 1 summarizes available single-agent data for DVA in patients with non-small cell lung cancer (NSCLC). Accordingly, DVA was evaluated in carefully selected patients with NSCLC.

PATIENTS AND METHODS

From September 1978 to February 1980, 33 patients with NSCLC were treated with DVA. There were 32 males and one female, with a median age of 58 years (range, 37–81). All patients had a pathologically confirmed diagnosis. The patient population is described in table 2.

Entry in the study required normal hematologic function, a Karnofsky performance status ≥ 50%, and measurable disease on a standard posteroanterior and lateral chest X-ray and/or physical examination. All patients were fully recovered from the toxicity of prior treatment. Baseline liver and renal function was assessed. Bone, liver, and brain scans were obtained as indicated. Informed consent was obtained from all patients.

DVA was given at a dose of 3 mg/m²/week by iv push. Doses were escalated by 0.5 mg/m² every fourth week to a maximum of 4 mg/m² if no significant toxic reactions occurred. Treatment was delayed 1 week and/or a 25% dose reduction was made for severe hematologic toxic effects (platelet count < 50,000/mm³ or wbc count < 2000/mm³). Neuromuscular toxicity could also necessitate a 25% dose reduction or a change from a weekly to an every-other-week schedule.

A complete remission (CR) was defined as the disappearance of all signs and symptoms of disease. A partial remission (PR) was defined as a ≥ 50% reduction in the sum of the products of perpendicular diameters

TABLE 1.—Results of DVA therapy in NSCLC

Authors (ref No.)	No. of patients	No. of responses (%)
Gralla et al (4)	46	10 (22)
Mattson et al (5)	37	4 (11)
Grundtvig et al (6)	34	8 (24)
Furnas et al (present study)	29	8 (28)

[1]Received Nov 19, 1980; revised June 9, 1982; accepted June 16, 1982.
[2]Supported in part by Public Health Service grant CA-19657 (Southeastern Cancer Study Group) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, and by a grant from Eli Lilly and Company. Dr. Einhorn is an American Cancer Society Clinical Professor of Oncology.

[3]Department of Medicine, Indiana University Medical Center, and the Indianapolis Veterans Administration Medical Center.
**Reprint requests to:* Lawrence H. Einhorn, MD, Indiana University Medical Center, University Hospital, Rm 582-N, 1100 W Michigan St, Indianapolis, IN 46223.

TABLE 2.—Response to DVA therapy

	No. of patients	No. of responses
Cell type		
Squamous cell carcinoma	15	1 CR, 4 PRs (33%)
Adenocarcinoma	8	3 PRs (38%)
Large cell carcinoma	3	0
Undifferentiated carcinoma	3	0
Karnofsky performance status		
80%–100%	22	6 (27%)
50%–70%	7	2 (29%)
Prior therapy		
None	16	3 PRs
Surgery	2	1 PR
Irradiation	7	1 CR, 2 PRs
Chemotherapy	3	0
Surgery + irradiation	1	0
Surgery + irradiation + chemotherapy	1	0
Irradiation + chemotherapy	2	0
Whole-brain irradiation + chemotherapy	1	1 PR
Prior vinca alkaloid therapy		
Yes	1	0
No	28	8

of measurable disease. All responses lasted a minimum of 4 weeks. Evaluable patients received at least two weekly doses of DVA.

Twenty-nine of the 33 patients were evaluable for response. Three patients refused further therapy after receiving one dose. A fourth patient had obstructive atelectasis with pneumonia and started receiving radiation therapy after two doses of DVA. Two of the 33 patients presented with brain metastases and received whole-brain radiation therapy as well as DVA.

Patients were followed with weekly cbcs and neurologic examinations. Tumor measurements as well as liver and renal function studies were repeated monthly.

RESULTS

Eight patients responded to DVA, as shown in table 2. Three of eight adenocarcinoma patients had PRs. Four PRs and one CR were achieved in 15 patients with squamous cell carcinoma; the CR was documented by whole-lung tomography. All eight responses were documented radiographically on chest X-ray, and one patient had a concomitant 75% decrease in a metastatic supraclavicular node as well. The overall response rate for the 29 evaluable patients was 28%. The median time to remission was 4.5 weeks (range, 1–8). The PRs lasted 4–18 weeks (median, 12); the CR lasted 26 weeks. The small number of patients in this study precludes drawing conclusions regarding the influence of disease site, cell type, performance score, or prior therapy on response. However, only one of seven patients who had received prior chemotherapy responded.

Two of the responding patients are alive at 8 and 15 months after DVA treatment. Three of the nonresponding patients remain alive at 13, 14, and 15 months after DVA. All patients currently have progressive disease. Median survival was 34 weeks for the responders and 24 weeks for the nonresponders.

Seven patients had from one to four doses of DVA omitted due to leukopenia (range, 800–2400 wbcs/mm^3). Two of the seven patients developed granulocytopenic infections that responded to antibiotics. On all but two occasions the wbc count had returned to acceptable levels within 1 week. Sustained myelosuppression was not seen.

Six patients had hemoglobin drops of 4.0–5.8 g/100 ml. None of the six patients required transfusion. Thrombocytopenia was not observed.

Alopecia developed in 15 of 22 patients evaluable for hair loss. The degree of alopecia appeared to be dose-related. Extravasation of DVA occurred on four occasions and resulted in a minor ulceration once.

Neurologic toxicity, the most frequent and dose-limiting side effect, was manifested by a variety of signs and symptoms. Peripheral neuropathy and generalized malaise occurred in 20 patients. Ten patients had scheduled dose increases delayed, doses decreased, or treatments changed to every other week due to neurotoxicity. Five patients (including four responders) who experienced difficulty walking or completing other motor skills ultimately had therapy terminated due to these toxic reactions. Moderate constipation occurred in five patients. One other patient was admitted to the hospital for a bowel obstruction. This did not resolve with conservative management, but at surgery the cause of ob-

struction was found to be adhesions. Blurred vision, jaw pain, and headache were occasionally reported.

Three patients developed fever and chills on the evening of their DVA injections. A severe generalized allergic rash developed in one patient 3 days after his first DVA injection, which appeared again upon rechallenge with the drug. This patient achieved a PR after these two doses but therapy had to be terminated.

DISCUSSION

The response rates of this disease-oriented phase II trial are comparable to those of Gralla et al (4) (22%) and Grundtvig et al (6) (24%) and are somewhat higher than that seen in the series of Mattson et al (5) (11%). Overall, by combining the results in all four series, responses have been seen in 30 of 146 (21%) evaluable patients (4–6). Thus, there has been considerable experience in disease-oriented trials with DVA and it is among the most active agents in NSCLC (7,8). If only patients without prior chemotherapy are considered, the cumulative response rate is 23% (26 responses among 115 patients) (4–6).[4]

In two studies (present series and ref 4) no difference was noted according to cell type, but overall, eight of 61 (13%) patients with squamous cell carcinoma responded versus four of 20 (20%) with large cell carcinoma and 18 of 62 (29%) with adenocarcinoma. It is difficult to make dogmatic conclusions from these data, but we feel there is no major difference in responsiveness among cell types.

In our experience, neurotoxicity has been a major problem. It is possible that lower doses, longer intervals, or different schedules [ie, continuous infusion (9)] may reduce toxicity. We currently utilize DVA at a weekly dose of 3 mg/m^2 for a maximum of 6 consecutive weeks and then give the same dose of DVA every other week; this has substantially reduced the neurotoxicity.

The relative merit of DVA as opposed to the old vinca alkaloids is unknown. A cumulative response rate of 11% for vinblastine (7) has been seen, and in two trials of vincristine, responses were seen in two of 37 (5%) and six of 35 (17%) patients (10). Of the three agents, it is likely that DVA is the most active.

We conclude that DVA has significant and reproducible activity in NSCLC, and combination chemotherapy and combined modality trials are warranted.

REFERENCES

1. Dyke RW, and Nelson RL. Phase I anti-cancer agents vindesine (desacetyl vinblastine amide sulfate). Cancer Treat Rev 4:135-142, 1977.
2. Ochs S, and Worth R. Comparison of the block of fast axoplasmic transport in mammalian nerve by vincristine, vinblastine and desacetyl vinblastine amide sulfate (DVA). Proc Am Assoc Cancer Res and ASCO 16:70, 1975.
3. Bodey GP, and Freireich EJ. Initial clinical studies of vindesine (desacetyl vinblastine amide sulfate). Proc Am Assoc Cancer Res and ASCO 17:128, 1976.
4. Gralla RJ, Raphael BG, Golbey RB, et al. Phase II evaluation of vindesine in patients with non-small cell carcinoma of the lung. Cancer Treat Rep 63:1343–1346, 1979.
5. Mattson K, Holsti LR, Salmo M, et al. Vindesine in the treatment of bronchogenic carcinoma: preliminary results of two clinical trials. *In* Proceedings of the 11th International Congress of Chemotherapy and the 19th Interscience Conference on Antimicrobial Agents and Chemotherapy, 1979, vol 11, pp 1569–1570.
6. Grundtvig P, Osterlind K, Pedersen AG, et al. Phase II trial of vindesine in bronchogenic carcinoma of WHO types I, II and IV. *In* Proceedings of the Second World Conference on Lung Cancer, 1980, vol 2, p 230.
7. Livingston RB, and Carter SK. Single agents in cancer chemotherapy. New York, NY, IFI/Plenum, 1970, pp 279–297.
8. Selawry OS. Monochemotherapy of bronchogenic carcinoma with special reference to cell type. Cancer Chemother Rep Part 3, vol 4(2):177–188, 1973.
9. Yap H-Y, Blumenschein GR, Bodey GP, et al. Continuous 5-day infusion vindesine—improvement in therapeutic index in the treatment of refractory breast cancer. Proc Am Assoc Cancer Res and ASCO 21:408, 1980.
10. Holland JF, Scharlau C, Gailani S, et al. Vincristine treatment of advanced cancer: a cooperative study of 392 cases. Cancer Res 33:1258–1264, 1973.

[4]Mattson K, Holsti LR, Salmo M, et al. Clinical trials on vindesine in small cell and non-small cell carcinoma of the lung. Presented at the Symposium on Vindesine. Washington, DC, Oct 1979.

Studies of Baker's Antifol, Methotrexate, and Razoxane in Advanced Gastric Cancer: A Gastrointestinal Tumor Study Group Report[1,2]

H. W. Bruckner,* J. J. Lokich, and D. M. Stablein[2]

In this multi-institutional study of advanced gastric cancer, 73 patients were evaluable for response or survival. Patients were treated with either triazinate (Baker's antifol), standard-dose methotrexate, or ICRF-159 (razoxane). Objective responses were seen in four patients receiving Baker's antifol, in three receiving methotrexate, and in none receiving razoxane. Baker's antifol produced a median survival of 18 weeks and methotrexate and razoxane produced a median survival of 8 and 9 weeks, respectively. Seventy of the 73 patients entered in this study had been previously treated, most frequently with combination chemotherapy regimens containing 5-FU or doxorubicin. This study appears to demonstrate that Baker's antifol is an active drug for patients with advanced gastric cancer, according to both response and survival criteria. Examination of the pretreatment prognostic characteristics of the patients further suggests that a possible survival advantage is due to treatment with Baker's antifol rather than the prognostic characteristics of the patients. [Cancer Treat Rep 66:1713–1717, 1982]

Advanced gastric cancer has shown response to several cytotoxic drugs, most notably, 5-FU, mitomycin, and doxorubicin (1–8). Response rates have been in the 15%–30% range. There have been suggestions in the literature that drug combinations involving 5-FU and either mitomycin, nitrosoureas, or cytarabine may increase objective response rates (1–3,6,7). Gastrointestinal Tumor Study Group (GITSG) investigations, however, did not show or suggest a survival advantage for any of these drug combinations (8,9). These phase II–III studies have found a survival advantage for 5-FU and semustine or mitomycin in combination with doxorubicin when compared to combinations without doxorubicin (8,9).

The GITSG was previously able to confirm (5) that doxorubicin was an active drug for patients with advanced gastric cancer, both as primary and secondary therapy. Secondary therapy produced three responses among 17 patients (8). The phase II study reported herein initially compared triazinate (Baker's antifol), standard-dose methotrexate, ICRF-159 (razoxzane), and high-dose methotrexate with leucovorin calcium in similar patients with measurable disease failing prior chemotherapy. The concept of directly testing the antifolate compounds was scientifically attractive, and razoxane was of additional interest due to its reported ability to prevent doxorubicin toxicity and tumor angiogenesis in preclinical systems (10). However, the high-dose methotrexate regimen was suspended because it proved impractical to accrue patients. It will not be considered in the remainder of this report.

METHODS

All patients selected for the study were required to have known gastric adenocarcinoma with histologic proof of unresectable, residual, recurrent, or metastatic disease. All patients were required to have measurable disease with an area of known tumor that could be clearly calibrated. Palpable hepatomegaly was acceptable if the liver was proven to contain metastasis and if a well-defined liver edge extended at least 5 cm below either the xiphoid process or the costal margin on quiet respiration. A positive liver scan was accepted if there was a clearly defined perfusion defect measuring at least 5 cm in diameter. Lesions visualized only by contrast roentgenography or lesions for which size would only be estimated, eg, pelvic or extrarectal masses, were not accepted. Contraindications to entry in the study included the following: recent surgery with resection

[1]Received Jan 11, 1982; revised May 21, 1982; accepted June 7, 1982.

[2]Supported by contracts (CM) from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health and Human Services. Participating institutions and investigators include: Mayo Clinic, Rochester, MN (*C. G. Moertel, M. J. O'Connell, J. Rubin, A. J. Schutt*, N01-CM-43783); Mount Sinai School of Medicine, New York, NY (*H. W. Bruckner, B. Kabakow, J. A. Storch*, N01-CM-53841 and N01-CM-07407); Roswell Park Memorial Institute, Buffalo, NY (*H. O. Douglass, Jr., H. R. Nava, R. A. Berjian, S. Milliron, P. M. Sweeney*, N01-CM-43782 and N01-CM-07410); Wayne State University, Detroit, MI (*L. Leichman*, N01-CM-07408); Yale University, New Haven, CT (*E. M. Livstone, J. Marsh, H. K. Wright*, (N01-CM-53843 and N01-CM-07339); New England Deaconess Hospital, Boston, MA (*J. J. Lokich*, N01-CM-43781); and The EMMES Corporation, Potomac, MD (*D. M. Stablein*, N01-CM-87193).

**Reprint requests to:* H. W. Bruckner, MD, Mount Sinai School of Medicine, Department of Medicine, 1 Gustave L. Levy Pl, New York, NY 10029.

or bypass (< 3 weeks previously); recent abdominal exploration and biopsy only (< 2 weeks previously); recent radiation therapy or chemotherapy (< 4 weeks previously); total disability (Eastern Cooperative Oncology Group [ECOG] performance score of 4); severe malnutrition, severe nausea, or frequent vomiting; an active infectious process; and leukopenia (< 4000 cells/mm^3) or thrombocytopenia (< 150,000 cells/mm^3).

Patients were stratified according to initial performance status (ECOG scale of 0 or 1 vs 2 or 3); prior therapy with agents not used in the study vs prior razoxane vs prior antifolates; and serum bilirubin < 1.5 versus > 1.5 mg/100 ml. Patients were randomly assigned to the treatment arms, with the following exceptions: prior therapy with the study agent or its analog excluded patients from randomization to the arm containing the prior study agent; and patients with bilirubin > 1.5 mg/100 ml were not eligible for treatment with Baker's antifol.

Patients were required to have iv pyelograms and to have creatinine clearance > 70 ml/minute, serum creatinine < 1.5 mg/100 ml, or BUN < 25 mg/100 ml; they had to be ineligible for GITSG phase III combination therapy protocols either because of prior treatment with a study agent (5-FU, doxorubicin, or a long-acting alkylating agent) or because heart disease contraindicated doxorubicin. In practice, one institution did not perform creatinine clearance determinations. Patients were also considered to be ineligible if their caloric intake was < 1500 calories/day or if there was evidence of severe malnutrition, nausea or vomiting, or third-space fluid overload, eg, ascites, pleural effusion, or major edema. Patients were required to have provided informed consent in accordance with current institutional and federal regulations.

Baker's antifol was given as a 250-mg/m^2/day infusion over 1 hour for 3 consecutive days and was repeated every 3 weeks (Days 1–3, 22–24, etc). Standard-dose methotrexate was given as a 40-mg/m^2 bolus injection once weekly, and razoxane was given at a dose of 300 mg/m^2 three times a day orally for nine doses, every 4 weeks (Days 1–3, 29–32, etc).

For each regimen, weekly wbc and platelet counts were recommended, as well as blood chemistries and a comprehensive physical examination before each treatment course. Appropriate reductions in doses of subsequent courses were made if excessive toxicity was observed. For those patients with measurable disease, the criteria for objective response required all of the following: *(a)* reduction by at least 50% of the product of the longest perpendicular diameter of the most clearly measurable tumor lesion (if hepatomegaly was the indicator lesion, it was required that there be a reduction of the sum of measurements below the xiphoid and each costal margin by at least 30%); *(b)* no increase in the size of any other measurable lesion and no new lesions of malignant disease; and *(c)* no significant loss in body weight (> 5%) or deterioration of performance status (one level by ECOG score). The criteria for disease progression included: *(a)* an increase in the size of any measurable lesion by > 25%; *(b)* the appearance of new areas of malignant disease; and *(c)* a loss in body weight (> 5%) or the deterioration of performance status (one level by ECOG score).

RESULTS

The study was activated in September 1977. In September 1979, the group decided to keep each arm open until it accrued at least ten patients with an ECOG initial performance status of 0 or 1. In June 1980, accession to the razoxane arm was discontinued. It had accrued 19 patients without demonstrating antitumor activity.

At the conclusion of the study, 75 patients had been entered in the study and 73 patients were included in the analysis. Two patients, one from the Baker's antifol group and one from the standard-dose methotrexate group, never received protocol therapy (one refused participation and the other was declared ineligible prior to treatment initiation). Twenty-six patients received Baker's antifol, 28 received standard-dose methotrexate, and 19 received razoxane.

Patient characteristics are listed in table 1.

Most of the patients had a poor performance status (ECOG of 2 or 3). Only one patient from each of the completed study arms was previously untreated, two due to heart disease. Patient characteristics included: 90% with serum bilirubin ≤ 1.5 mg/100 ml and 15% with linitis plastica histology. The modal patient treated with razoxane or Baker's antifol had poorly differentiated tumors, and the modal patient treated with methotrexate had moderately differentiated tumors. There was a 3:1 male predominance; one-half of the patients had lost ≥ 5% of their body weight within the previous 6 months, and one-half had demonstrable metastasis to the liver. The median time from initial diagnosis to protocol treatment was 43 weeks with Baker's antifol, 37 weeks with methotrexate, and 28 weeks with razoxane, a significant difference ($P = 0.01$). The median ages also nearly differ statistically ($P = 0.055$); the median age of the patient who received Baker's antifol was 63 years as opposed to 55 years for the patients who received razoxane. The distribution of the patients' on-study performance status significantly differed ($P = 0.01$) among the participating institutions. One institution contributed 73% of the patients with ECOG performance status of 0 or 1.

Toxicity

Thirty-two patients experienced severe or worse treatment-related toxic effects. Of these patients, ten

TABLE 1.—Selected patient characteristics (GI-8477)

Characteristic	Treatment			
	Baker's antifol	Standard-dose methotrexate	Razoxane	Total
No. of patients	26	28	19	73
ECOG performance score				
0	3	1	1	5
1	9	10	6	25
2	8	11	7	26
3	6	6	5	17
Prior therapy				
No study agents	22	23	17	62
Razoxane	4	5	0	9
Antifolates	0	0	2	2
Bilirubin (mg/100 ml)				
≤ 1.5	26	25	15	66
> 1.5	0	3	4	7
Primary type				
Linitis plastica	6	4	1	11
Other	20	24	18	62
Differentiation				
Good	4	3	0	7
Moderate	8	15	5	28
Poor	11	10	13	34
Unknown	3	0	1	4
Sex				
Male	18	22	15	55
Female	8	6	4	18
Weight loss				
< 5% of body weight	11	8	9	28
≥ 5% of body weight	13	17	10	40
Unknown	2	3	0	5
Lung involvement				
Yes	5	6	6	17
No	19	21	10	50
Unknown	2	1	3	6
Liver involvement				
Yes	12	17	9	38
No	10	6	8	24
Unknown	4	5	2	11
Median age in yrs	63	63	55	61
Median time in wks from initial diagnosis to treatment	43	37	28	38

were receiving Baker's antifol, ten were receiving standard-dose methotrexate, and 11 were receiving razoxane. One toxic death was observed in a patient receiving standard-dose methotrexate. There were no apparent imbalances in the overall number of patients experiencing severe toxic effects in these three treatments. As expected, Baker's antifol produced some instances of severe stomatitis and body rash, and standard-dose methotrexate produced anorexia, stomatitis, and one episode of moderate renal failure (serum creatinine, 2.7 mg/100 ml). These were known potential complications of the antifolate regimens. All 11 patients experiencing severe toxicity on the razoxane arm had decreased wbc or platelet counts. Only four patients with severe myelosuppression were seen in both the Baker's antifol and standard-dose methotrexate arms. Razoxane was significantly ($P < 0.01$) more myelosuppressive by these criteria at the study's dosages.

Failure to measure creatinine clearance before randomization had no apparent impact on toxicity. Nevertheless, determination of creatinine clearance may be a useful safeguard before deciding on therapy with methotrexate.

Tumor Response To Therapy

Two partial responses and one complete response were observed in patients treated with standard-dose methotrexate. Four partial responses were observed in patients treated with Baker's antifol. Responses included complete remission of pulmonary metastases (methotrexate) and partial remission of abdominal masses (both methotrexate and Baker's antifol), liver metastases (Baker's antifol), and subcutaneous nodules (Baker's antifol).

If patients with early deaths (within 21 days) without tumor progression are removed from the denominator, the response rates are 17% (four responses among 23 patients), 12% (three responses among 25 patients), and 0% (no response among 17 patients) for Baker's antifol, methotrexate, and razoxane, respectively. If all patients are considered, response rates are 15% (four responses among 26 patients), 11% (three responses among 28 patients), and 0% (no response among 15 patients) for the same three drugs, respectively.

Response rates by ECOG status for Baker's antifol and methotrexate are 25% (one response among four patients), 21% (four responses among 19 patients), 5% (one response among 19 patients), and 8% (one response among 12 patients) for ECOG performance status 0, 1, 2, and 3, respectively. Only one response was reported from institutions that predominantly contributed patients with poor performance status.

Only 18% of the patients had responded to prior therapy. Only two responders, one on each arm, had responded to prior therapy.

Sixty-eight deaths have been observed among the 73 patients reported herein. The estimated median survival times are: 18, 9, and 8 weeks for patients treated with Baker's antifol, standard-dose methotrexate, and razoxane, respectively. The product limit estimates of the survival curves are shown in figure 1. A comparison of the three survival distributions, using both the log-rank and Gehan-Wilcoxon tests, found a difference among the three treatments ($P < 0.05$). Poor performance status (2 or 3) and substantial weight loss ($\geq 5\%$) in the previous 6 months are both significantly associated with poor prognosis ($P < 0.01$). Neither age nor time interval from diagnosis to protocol treatment influenced prognosis or affected treatment comparisons. Pair-wise comparisons of the treatment arms again demonstrate the superiority of Baker's antifol to the other treatments after adjustment for the performance status and weight loss ($P < 0.05$). Removal of patients with high

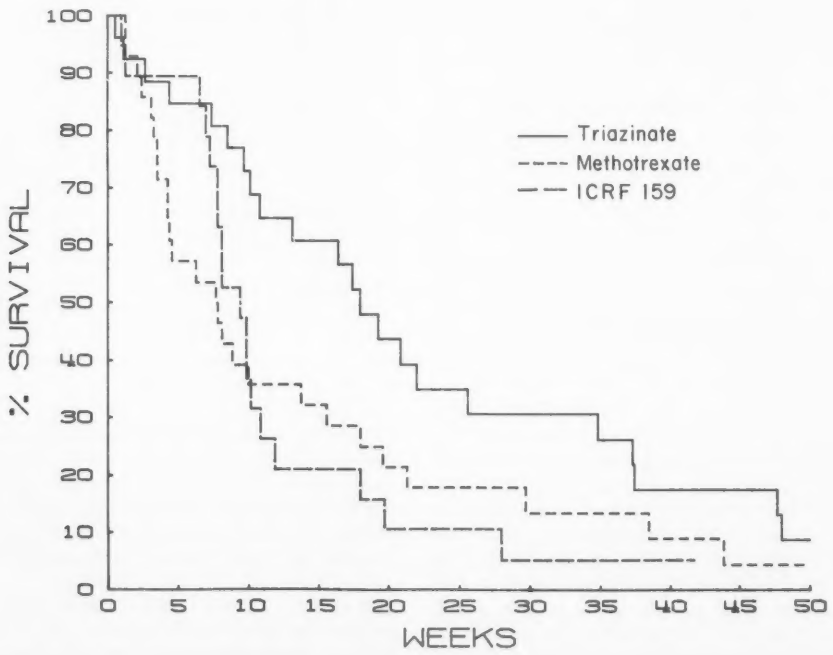


FIGURE 1.—Product limit estimates of survival curves for patients treated with Baker's antifol (triazinate), methotrexate, or razoxane (ICRF 159).

bilirubin levels (> 1.5 mg/100 ml), who were excluded by design from the Baker's antifol arm, minimally affected the significance of pair-wise tests. One-tailed tests maintained their significance. Performance status by institution did not affect the observed differences in survival by treatment.

Further analyses examined the type, duration, and results of prior chemotherapy in relation to response and survival. There were no imbalances with respect to the methotrexate and Baker's antifol groups.

DISCUSSION

The study found that Baker's antifol produces objective tumor responses and has an apparent beneficial impact on overall survival compared to standard-dose methotrexate or razoxane for patients with advanced measurable gastric carcinoma refractory to treatment with 5-FU, doxorubicin, and long-acting alkylating agents. Response rates for Baker's antifol are comparable to those previously observed with doxorubicin in patients failing prior therapy (8,11). These observations provide the rationale for advancing Baker's antifol into phase III combination therapy trials. Additional investigation on this and other antifolates including methotrexate appears warranted both to confirm these observations and to test other dose schedules.

The observed 18-week median survival following treatment with Baker's antifol appears relatively superior to that with standard-dose methotrexate or razoxane and comparable to that with doxorubicin in similar second-line treatment studies (5,8). The observed impact of Baker's antifol on survival was supported by survival comparisons, with adjustment for potentially important prognostic variables.

The apparent new findings of this study must be viewed cautiously. The survival advantage by treatment appeared to overcome prognostic considerations; however, the initial "randomization" was conditional. In theory, unrecognized factors may be responsible for a false-positive survival analysis. The finding that a second antifolate also produces objective responses would appear to support the potential efficacy of both antifolates. The failure to find an overall survival advantage for patients treated with methotrexate led the GITSG to select Baker's antifol for phase III study.

With this study, the GITSG has a positive report suggesting a role for chemotherapy in every stage of gastric cancer (8,9,12–14). Patients with a response to chemotherapy were found to have improved survival twice that of all patients with the same profile of prognostic characteristics (12). The 5-FU-semustine regimen, which was found to have no impact on survival with advanced disease, appears to improve survival in early stages of disease (13,14). These findings across all stages of gastric cancer strongly warrant continued systematic testing of new combinations and new drugs for patients with advanced gastric cancer.

REFERENCES

1. Reitemeir RJ, Moertel CG, and Hahn RG. Combination chemotherapy in gastrointestinal cancer. Cancer Res 30:1425–1428, 1970.
2. Kovach JS, Moertel CG, Schutt AJ, et al. A controlled study of combined 1,3-bis-(2-chloroethyl)-1-nitrosourea and 5-fluorouracil therapy for advanced gastric and pancreatic cancer. Cancer 33:563–567, 1974.
3. Moertel CG, Mittelman JA, Bakemeier RF, et al. Sequential and combination chemotherapy of advanced gastric cancer. Cancer 38:678–682, 1976.
4. Moertel CG, Engstrom P, Lavin PT, et al. A controlled chemotherapy of gastric and pancreatic carcinoma evaluation of combinations of 5-fluorouracil with nitrosoureas and "lactones." Surgery 85:509–513, 1979.
5. Frytak S, Moertel CG, Schutt AJ, et al. Adriamycin (NSC-123127) therapy for advanced gastrointestinal cancer. Cancer Chemother Rep 50:405–409, 1975.
6. Ota K, Kurita S, and Nishimura M. Combination chemotherapy with mitomycin C (NSC-26980), 5-fluorouracil (NSC-19893), and cytosine arabinoside (NSC-63878) for advanced cancer in man. Cancer Chemother Rep 56:373–385, 1972.
7. De Jager R, Magill GB, Golbey RB, et al. Mitomycin C, 5-fluorouracil and cytosine arabinoside (MFC) in gastrointestinal cancer. Proc Am Assoc Cancer Res and ASCO 15:178, 1974.
8. The Gastrointestinal Tumor Study Group. Phase II–III chemotherapy studies in advanced gastric cancer. Cancer Treat Rep 63:1871–1876, 1979.
9. Gastrointestinal Tumor Study Group. A comparative clinical assessment of combination chemotherapy in the management of advanced gastric carcinoma. Cancer. In press.
10. Wasserman TH, Friedman MA, Slavik M, et al. Clinical Brochure. ICRF-159 (NSC-129943). Bethesda, MD, National Cancer Institute, Oct 1973.
11. Moertel CG, and Lavin PT. Phase II–III chemotherapy studies in advanced gastric cancer. Cancer Treat Rep 63:1863–1869, 1979.
12. Bruckner HW, Lavin PT, Plaxe S, et al. Objective laboratory test as prognostic determinates for the analysis of gastric cancer trials (for the Gastrointestinal Tumor Study Group). Proc Am Assoc Cancer Res and ASCO 21:180, 1980.
13. Gastrointestinal Tumor Study Group. A comparison of combination chemotherapy and combined modality therapy for locally advanced gastric carcinoma. Cancer 49:1777, 1982.
14. Douglass HO, Novak J, Bruckner HW, et al. Controlled trial of adjuvant chemotherapy following curative resection of gastric cancer. The Gastrointestinal Tumor Study Group. *In* Adjuvant Therapy of Cancer III (Jones SE, and Salmon JE, eds). Grune & Stratton, Inc, pp 503–510, 1981.

Epileptic Seizures Associated With Cisplatin Administration[1]

Graham M. Mead,* Andrew M. Arnold, John A. Green, Fergus R. Macbeth, Christopher J. Williams, and J. Michael Whitehouse[2]

Eighty-one patients with germ cell tumors or ovarian carcinoma were treated over a 4-year period with cisplatin-containing combination chemotherapy. Eight patients (9.8%) experienced epileptic seizures during or within 3 months of this chemotherapy. While a definite causal relationship between the seizures and cisplatin could not be established, we have rarely seen such episodes in relationship to other cytotoxic drugs. A number of possible contributing factors were apparent, including hyponatremia and pretreatment impairment of renal function. [Cancer Treat Rep 66:1719–1722, 1982]

Cisplatin is a widely used chemotherapeutic agent that has shown particular activity in nonseminomatous germ cell tumors and ovarian carcinoma (1,2). However, toxic effects, particularly nausea and vomiting and progressive impairment of renal function, are common (3). In addition, ototoxicity [manifest by high-tone hearing loss (4)], myelosuppression (3), and renal electrolyte wasting (particularly of Mg^{++} and Ca^{++}) are well-described (5,6). Neurologic toxicity has predominantly comprised sensory and motor peripheral neuropathy (7–12), although there are reports of seizures associated with use of the drug (12–15).

This report summarizes our experience with seizures related to cisplatin-containing combination chemotherapy and attempts to identify contributory factors in their etiology.

MATERIALS AND METHODS

Hospital charts were reviewed on all patients receiving cisplatin between August 1977 and August 1981. This drug was used exclusively to treat metastatic carcinoma of the ovary (55 patients) and germ cell tumors of the testis and ovary (26 patients). A total of 298 treatment courses were given (one to eight per patient), all in combination with other drugs. All patients were in the hospital for 24–48 hours to receive their treatment.

Chemotherapy for ovarian carcinoma (PACe) comprised cisplatin (80 mg/m^2), doxorubicin (40 mg/m^2), and cyclophosphamide (1 g/m^2), all given by iv push on Day 1 of a 28-day cycle. Cisplatin administration was preceded by an infusion of 5% dextrose in $^1/_5$ normal saline given iv at a rate of 500 ml every 2 hours for at least 2 hours and until the urine output exceeded 150 ml/hour. Cisplatin was reconstituted in water for injection. Iv fluids were continued for at least 6 hours, alternating 500 ml of normal saline with 500 ml of 5% dextrose in $^1/_5$ normal saline with potassium supplements. Iv fluids were then continued every 3 hours until emesis stopped and the patients were able to drink adequately. Doses of all drugs were modified according to peripheral blood cell counts prior to therapy. Cisplatin doses were modified according to renal function; the drug was withheld if the creatinine clearance was < 35 ml/minute, a 50% dose was given for a clearance of 35–50 ml/minute, and a 100% dose was given for a clearance > 50 ml/minute. These guidelines were sometimes contravened if cisplatin administration was felt to be clinically indicated.

Patients with germ cell tumors were treated with either PVB chemotherapy as described by Einhorn and Williams (16) or a modification of this therapy. PVB chemotherapy comprised cisplatin (20 mg/m^2) iv daily for 5 days, vinblastine (0.15–0.2 mg/kg) iv on Days 1 and 2, and bleomycin (15 mg/m^2) iv on Days 2, 9, and 16 of a 21-day cycle. Early in our experience with this therapy, cisplatin was given as a 100-mg/m^2 iv bolus on Day 1. A number of patients, treated as part of a national study, received etoposide at a dose of 100 mg/m^2 iv daily for 5 days in conjuction with PVB. When this regimen (BEViP) was used, the vinblastine dose was reduced to 0.15 mg/kg on Days 1 and 2. Hydration prior to cisplatin administration was as for the PACe regimen.

Patients receiving cisplatin over a 5-day period were given an iv infusion of normal saline (500 ml every 4 hours with potassium supplements) throughout the treatment period. Dose modifications for nephrotoxicity were as described for PACe chemotherapy.

Hospital charts were carefully reviewed for evidence of neurotoxicity possibly related to cisplatin administration. Neurologic events occurring within a 6-month period of drug administration were recorded and analyzed. The subsequent course of the patient was assessed to exclude, as far as possible, metastatic disease as an explanation for these events.

[1]Received Feb 11, 1982; revised May 24, 1982; accepted June 7, 1982.

[2]CRC Medical Oncology Unit, Southampton General Hospital, England.

**Reprint requests to:* CRC Medical Oncology Unit, Southampton General Hospital, Southampton, SO9 4XY, England.

RESULTS

Neurologic Events

Eight patients (9.8%) had epileptic seizures possibly related to cisplatin therapy. In addition, two of these patients (Nos. 3 and 8) reported numbness and paresthesia in their extremities after completing chemotherapy, which was consistent with peripheral sensory neuropathy.

A further six patients (7.4%), comprising five who had ovarian carcinoma treated with PACe and one who had germ cell tumor of the testis treated with PVB, described sensory symptoms consistent with peripheral neuropathy. These symptoms occurred after therapy was completed, were not associated with motor weakness, and disappeared spontaneously within 6 months. Finally, two patients (one with ovarian carcinoma receiving PACe and one with germ cell tumor of the testis receiving PVB) had Lhermitte's syndrome following completion of therapy; the symptoms lasted approximately 3 months.

Seizures

Details of the patients who had seizures are recorded in table 1. There were five female patients (ovarian carcinoma, four; germ cell tumor of the ovary, one) and three male patients (all with germ cell tumors of the testis). Their ages ranged from 23 to 66 years.

The temporal relationship of seizures to therapy was widely variable. One patient (No. 1) developed involuntary movement of his left hand and arm during the first drug infusion. These episodes recurred on several occasions within 7 days of the first and third drug cycles. Other patients developed seizures within 8 hours, 2 days, 7 days, 2 months, and 3 months of cisplatin administration. Although seizures occurring several weeks after drug therapy cannot necessarily be attributed to cisplatin, we have been impressed that such problems occur rarely in a much larger series of patients receiving other chemotherapy regimens.

Seizures were not necessarily considered a contraindication to further cisplatin therapy, particularly if easily identifiable precipitating factors were apparent. Thus, three patients (Nos. 1–3) received additional drug despite a history suggestive of seizures early in their therapy. Patients 1 and 2 developed probable evidence of seizure activity related to two of four and three of four PVB courses, repsectively. Patient 2 was ultimately admitted to the hospital in status epilepticus.

Seizures were clearly focal in four patients (Nos. 1, 2, 5, and 7) and were associated with transitory postictal evidence of mild hemiparesis in three (Nos. 2, 5, and 7). A single patient (No. 2) has continued to have an occasional grand mal seizure and has required continued treatment with phenytoin. There were otherwise no permanent neurologic sequelae.

Investigation of Seizures

All patients who had seizures were immediately tested for abnormalities of electrolytes (including serum Ca^{++}), renal function, and blood glucose. Serum magnesium concentrations were not routinely evaluated.

No neurologic investigations were carried out in five patients, since seizures occurred only once and precipitating factors were recognizable. Two patients (Nos. 2 and 7) had head CT scans and one patient (No. 4) had an isotope brain scan with normal results. These same patients also had an EEG examination; for two (Nos. 2 and 7), an epileptic focus was found (table 1).

Precipitating Factors

In seven patients, factors that may have contributed to an epileptic tendency were apparent. These included an association with micturition and recent phenothiazine administration (patient 3; probable diagnosis of micturition syncope), probable hypoxia in a patient (No. 4) disa led by bleomycin-induced lung fibrosis, and moderately severe hyponatremia (patients 6 and 8). This was related in one case to a recent abdominal paracentesis and in both cases to severe emesis after cisplatin administration. Mild impairment of renal function was apparent before therapy in three patients (Nos. 5, 7, and 8): two developed seizures early (6 hours and 2 days after treatment) and one developed seizures after 3 months.

Another possible contributing factor was moderate thrombocytopenia at the time of a seizure (patient 5). Finally, patient 2 was receiving anticoagulants for pulmonary emboli, and at the time of admission she had evidence of a bleeding diathesis (BCR, 6.1; anticoagulant range, 2–4) and hypertension. There was no evidence of peripheral bleeding.

No clear evidence of a cumulative dose effect was apparent.

Clinical Outcome

All four patients with germ cell tumors are alive and free of tumor at 4+, 20+, 28+, and 31+ months after completion of induction therapy. Patient 6 with ovarian carcinoma died of progressive abdominal disease within 2 months of her final cisplatin therapy (no autopsy was done), and the other three patients are alive with disease at 4+, 7+, and 14+ months.

DISCUSSION

Cisplatin has proved to be a highly successful cytotoxic drug, particularly in the therapy of germ cell tumors and ovarian carcinoma (1,2). Unfortunately, a

TABLE 1.—Characteristics of 8 patients who had seizures during or after cisplatin-containing therapy

Patient No.	Age and sex	Diagnosis*	Therapy	Seizure description	Temporal relationship to therapy	Possible precipitating factors	Neurologic investigations	Outcome†
1	23 M	Stage III NSGCT	BEViP × 4	Multiple focal seizures of left hand and arm; single grand mal episode	During and up to 7 days after chemotherapy courses 1 and 3	—	—	No seizures since completed chemotherapy; $A^0$20
2	34 F	Stage III germ cell tumor of ovary	PVB × 4	1. Unexplained blackouts × 2 while recumbent 2. Focal seizures of left arm + grand mal seizures; mild left hemiparesis	1. 7 days after courses 1 and 3 2. 7 days after course 4	At time of focal seizures: 1. Receiving anticoagulant Coumadin for pulmonary emboli On admission: 2. Blood pressure 210/130 3. BUN 96 mg/100 ml	Head computerized tomographic (CT) scan normal; EEG focus right posterior temporal region	Complete recovery though occasional grand mal seizure while receiving phenytoin; $A^0$31
3	36 M	Stage III NSGCT	PVB × 4	Single grand mal seizure	7 hrs after PVB, course 3	1. Chlorpromazine im 2 hrs before seizure 2. Seizure occurred after standing to micturate 3. Postural hypotension observed	—	No further seizures; symptoms of peripheral neuropathy; $A^0$4
4	38 M	Stage II NSGCT	PVB × 5	Grand mal seizures × 2	3 and 4 mos after PVB completion	Bleomycin-induced lung fibrosis with hypoxia on exertion	Isotope brain scan normal; EEG normal	No further seizures; $A^0$28
5	55 F	Stage III ovarian carcinoma	PACe × 3	Grand mal and focal seizures involving left side; mild left hemiparesis	2 mos after PACe, course 3	On admission: 1. Platelet count 60×10^9/mm^3 2. BUN 60 mg/100 ml	—	Complete recovery, no further seizures; A^+14
6	61 F	Stage III ovarian carcinoma	PACe × 2	Grand mal seizures	2 days after PACe course 2	1. Abdominal paracentesis prior to chemotherapy (electrolytes normal at this time) 2. Serum Na^+ 109 mg/100 ml at time of seizures	—	Progressive disease; D^+2
7	64 F	Stage III ovarian carcinoma	PACe × 4	Grand mal and focal right-sided seizures; mild right hemiparesis + dysphasia	6 hrs after PACe, course 4	1. BUN 84 mg/100 ml and creatinine 1.2 mg/100 ml prior to chemotherapy due to right uteric obstruction	Head CT scan, cerebral atrophy, EEG-focus left posttemporal region	Complete recovery, no further seizures; A^+4
8	66 F	Stage III ovarian carcinoma	PACe × 3	Grand mal seizure	2 days after PACe, course 3	1. BUN 50 mg/100 ml and creatinine 1.3 mg/100 ml prior to chemotherapy 2. Serum Na^+ 112 mg/100 ml at time of seizure	—	No further seizures, symptoms of peripheral neuropathy; A^+7

*NSGCT = nonseminomatous germ cell tumor.
†A^0 = alive with no clinical evidence of disease; A^+ = alive with clinical evidence of disease; and D^+ = dead with evidence of disease. Values = No. of mos.

number of toxic effects are common (1–15) and make the drug intolerable to some patients.

Toxicity to the nervous system is now well-described and is commonly manifested by sensory peripheral neuropathy, occasionally progressing to motor involvement (7–12). Figures indicating the incidence of this complication are not available; however, subclinical evidence of nerve damage with increasing dose has not been uncommon in prospective nerve conduction studies (8).

Other reports of neurologic damage have included rare patients who have developed papilledema (10,17), retrobulbar neuritis (17), low pressure hydrocephalus (10), and transient cortical blindness (15).

An association between cisplatin administration and seizures has been alluded to in a number of studies (12–15), although few detailed case reports are available. The series reported in this study is the largest in the literature, with almost 10% of the patients who received cisplatin developing clinical evidence of seizures within 3 months of therapy. It is possible that our rather broad inclusion criteria were partly responsible for this high incidence.

Close examination revealed a number of possible precipitating factors, some of which may have entirely accounted for the seizure activity. In two of our cases moderately severe treatment-related hyponatremia (known to be a cause of seizures) was present. In four cases there was evidence of impairment of renal function either at the time of cisplatin therapy or subsequently when seizures developed. Although insufficient to explain the seizures, it is possible that high peak levels or prolonged CNS exposure to this drug lowers the threshold at which these events might occur.

None of the other chemotherapeutic drugs used in these patients has been described as causing seizures. We were unable to demonstrate evidence of cerebral metastases either at the time of evaluation or at followup of these patients. Serum magnesium levels were not routinely evaluated. Although hypomagnesemia resulting from cisplatin administration has been well-described (5,6), seizures as a result of this complication have not been reported. Nevertheless, hypomagnesemia may have been a contributing factor (18,19) and should be looked for in this setting.

In most of our patients, seizures occurred only once and did not require long-term antiepileptic therapy. We have not regarded only one seizure as a contraindication to further therapy with cisplatin, although this decision must be taken on an individual patient basis in conjunction with other clinical factors.

In conclusion, we have described an association between the administration of cisplatin and the development of seizures in patients with germ cell tumors and carcinoma of the ovary. Seizures have occurred at any time from 6 hours to 3 months after therapy and although no definite causative relationship could be established it does seem likely that cisplatin lowered the seizure threshold. In some cases this may be sufficient to precipitate seizures, while in others the coexistence of other factors may be necessary. This study indicates that careful attention to electrolyte balance, particularly where vomiting is severe or protracted, is most important. Additionally, moderate impairment of renal function may have contributed to the possibility of seizures and should be regarded as a further relative contraindication to further use of the drug in this setting.

REFERENCES

1. Rozencweig M, Von Hoff DD, Slavik M, et al. *cis*-Diamminedichloroplatinum (II):a new anticancer drug.Ann Intern Med 86:803–812, 1977.
2. Williams CJ, and Whitehouse JMA. Cis-platinum: a new anticancer agent. Br Med J 1:1689–1691, 1979.
3. Von Hoff DD, Schilsky R, Reichert CM, et al. Toxic effects of *cis*-dichlorodiammineplatinum(II) in man. Cancer Treat Rep 63:1527–1531, 1979.
4. Reddell RR, Kefford RF, Grant JM, et al. Ototoxicity in patients receiving cisplatin: importance of dose and method of drug administration. Cancer Treat Rep 66:19–23, 1982.
5. Blachley JD, and Hill JB. Renal and electrolyte disturbances associated with cisplatin. Ann Intern Med 95:628–632, 1981.
6. Schilsky RL, and Anderson T. Hypomagnesaemia and renal magnesium wasting in patients receiving cisplatin. Ann Intern Med 90:929–931, 1979.
7. Hadley D, and Herr HH. Peripheral neuropathy associated with *cis*-dichlordiammineplatinum treatment. Cancer 44:2026–2028, 1979.
8. Cowan JD, Kies MS, Roth JL, et al. Nerve conduction studies in patients treated with *cis*-diamminedichloroplatinum(II): a preliminary report. Cancer Treat Rep 64:1119–1122, 1980.
9. Kedar A, Cohen ME, and Freeman AI. Peripheral neuropathy as a complication of *cis*-dichlorodiammineplatinum(II) treatment: a case report. Cancer Treat Rep 62:819–821, 1978.
10. Ostrow S, Egorin MJ, Hahn D, et al. *cis*-Dichlorodiammineplatinum and adriamycin therapy for advanced gynecological and genitourinary neoplasms. Cancer 46:1715–1721, 1980.
11. Marin AC, and Rierson B. Peripheral neuropathy secondary to *cis*-dichlorodiammineplatinum (II). Ariz Med 36:898–899, 1979.
12. Wiltshaw E, Subramarian S, Alexopoulos C, et al. Cancer of the ovary: a summary of the experience with *cis*-dichlorodiammineplatinum at The Royal Marsden Hospital. Cancer Treat Rep 63:1545–1548, 1979.
13. Bruckner HW, Cohen CJ, Kabakow B, et al. Combination chemotherapy of ovarian carcinoma with platinum: improved therapeutic index. Proc Am Assoc Cancer Res and ASCO 18:339, 1977.
14. Rossof AH, Talley RW, Stephens R, et al. Phase II evaluation of *cis*-dichlorodiammineplatinum(II) in advanced malignancies of the genitourinary and gynecologic organs: a Southwest Oncology Group study. Cancer Treat Rep 63:1557–1564, 1979.
15. Berman IJ, and Mann MP. Seizures and transient cortical blindness associated with cis-platinum (II) diamminedichloride (PDD) therapy in a thirty year old man. Cancer 45:764–766, 1980.
16. Einhorn LH, and Williams SD. Chemotherapy of disseminated testicular cancer. A random prospective study. Cancer 46:1339–1344, 1980.
17. Ostrow S, Hahn D, Wiernik PH, et al. Ophthalmologic toxicity after *cis*-dichlorodiammineplatinum(II) therapy. Cancer Treat Rep 62:1591–1594, 1978.
18. Kruse HD, Orent ER, and McCollum EV. Studies on magnesium deficiency in animals. I. Symptomatology resulting from magnesium deprivation. J Biol Chem 96:519–532, 1932.
19. Flink EB. Magnesium deficiency. Etiology and clinical spectrum. Acta Med Scand 467:125–137, 1980.

High-Dose Allopurinol Modulation of 5-FU Toxicity: Phase I Trial of an Outpatient Dose Schedule[1,2]

Thomas N. Campbell,[3,*] Stephen B. Howell,[4] Craig Pfeifle,[4] and Barbara A. House[3]

In an attempt to decrease the activation of 5-FU by normal cells relative to cancer cells, 20 patients with metastatic cancer were given 72 courses of 5-FU and allopurinol (HPP) in a phase I trial. 5-FU was given daily by iv bolus injection for 5 consecutive days every 4 weeks; HPP, 300 mg orally every 8 hours for 6 consecutive days, was started 24 hours before the first injection of 5-FU. HPP appeared to modulate 5-FU toxicity by allowing higher doses (18–21 mg/kg daily for 5 days) to be given. Unexpectedly, neurotoxicity was the dose-limiting toxicity; it was slowly reversible and manifested primarily as encephalopathy, with some patients also having cerebellar signs. Gastrointestinal and hematologic toxic effects were mild and infrequent. Because of the high incidence of neurotoxicity and low response rate, this program does not appear to offer any advantages over conventional dose schedules of 5-FU alone. [Cancer Treat Rep 66:1723–1727, 1982]

Allopurinol (HPP) given simultaneously with 5-FU has been shown to decrease 5-FU toxicity for cells in tissue culture (1), for rodents (2), and for man (3,4). Tumor response rates are proportional to the 5-FU dose rate (5). The rationale for the concurrent use of 5-FU and HPP is that if HPP selectively protects normal tissues, then higher dose rates of 5-FU could be tolerated, and greater tumor response rates would be anticipated.

5-FU is converted intracellularly to two active metabolites, 5-fluorodeoxyuridine monophosphate (FdUMP) and 5-fluorouridine triphosphate (FUTP). FdUMP is a potent inhibitor of thymidine synthetase, and FUTP is incorporated into several species of RNA, interfering with subsequent function (6,7). The first step in the formation of these active metabolites is the conversion of 5-FU to FUMP (fig 1). This can occur via either the action of orotidylate phosphoribosyltransferase (OPRTase) or the sequential action of uridine phosphorylase and uridine kinase. HPP is converted by xanthine oxidase to oxipurinol, which is phosphorylated to 1-oxipurinol-5′-monophosphate (8), a potent inhibitor of orotidylate decarboxylase (ODCase) (9). Inhibition of ODCase results in enhanced formation of orotidine, causing inhibition of OPRTase and increasing the intracellular concentration of orotic acid. Orotic acid effectively competes with 5-FU for OPRTase (10), thus decreasing the formation of FdUMP and FUTP via the OPRTase pathway. HPP can potentially increase the selectivity of 5-FU if normal tissues depend primarily on the OPRTase pathway and malignant tissues on the uridine phosphorylate-kinase pathway for activation of 5-FU. In addition, the activity of OPRTase has been shown to be higher in colonic adenocarcinoma cells that are sensitive to the cytotoxic effect of 5-FU than in surrounding normal colonic mucosal cells (11). Thus, HPP could potentially increase the selectivity of 5-FU if the HPP dose rate could be titrated to inhibit the activity of OPRTase in normal cells; then, those adenocarcinoma cells with excess OPRTase activity should still be able to convert 5-FU to FdUMP and FUTP.

Two phase I trials have been reported indicating that HPP can reduce the toxicity of 5-FU in man. Fox et al (3) observed three complete and three partial responses among 12 patients (ten with colorectal carcinoma and two with gastric carcinoma). Only six of their 20 patients had had previous chemotherapy. In contrast, Kroener et al (12) found no responses in 14 patients with colorectal carcinoma, 11 of whom had received prior 5-FU therapy. Both of these trials were conducted using a 5-day constant infusion of 5-FU, a schedule requiring hospitalization. Therefore, we conducted a phase I trial of HPP in combination with 5-FU administered as a single iv bolus for 5 consecutive days of each month. The aim of this trial was to determine whether concurrent treatment with HPP would permit escalation of the 5-FU dose commonly used in the outpatient setting.

[1]Received Nov 30, 1981; revised Ju. 1982; accepted June 28, 1982.

[2]Supported in part by Public Health Service grant CA-23100 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, and by the Clayton Foundation for Research, California Division. Dr. Howell is a Clayton Foundation investigator.

[3]Kaiser Permanente Medical Center, San Diego, CA.

[4]Departments of Medicine and Pharmacy, and the Cancer Center, University of California, San Diego at La Jolla.

**Reprint requests to:* Thomas N. Campbell, MD, 4647 Zion Ave, San Diego, CA 92120.

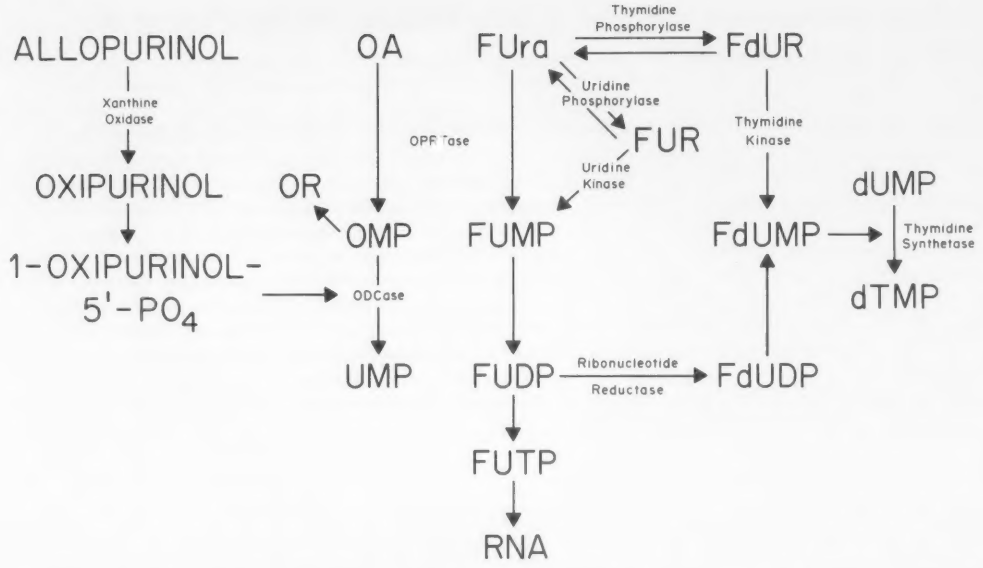


FIGURE 1.—Pathways of HPP, orotic acid, and 5-FU metabolism. OA = orotic acid; OMP = orotidine monophosphate; OR = orotidine; UMP = uridine monophosphate; FUra = 5-FU; FUR = 5-fluorouridine; FUMP = 5-fluorouridine monophosphate; FUDP = 5-fluorouridine diphosphate; FdUR = 5-fluorodeoxyuridine; FdUDP = 5-fluorouridine diphosphate; dUMP = deoxyuridine monophosphate; dTMP = thymidine monophosphate.

MATERIALS AND METHODS

Patients

Twenty adults with histopathologically proven advanced metastatic cancer, who were able to ingest oral medications, had recovered from toxic effects related to prior therapy, and had a life expectancy of at least 2 months, gave their informed consent to participate in this study. Twelve patients had colorectal adenocarcinoma; two had lung adenocarcinoma; one each had renal, adrenal, ovarian, breast, and pancreatic adenocarcinomas; and one had melanoma. Criteria for entry in the study included serum creatinine ≤ 1.5 mg/dl, leukocyte count $\geq 4000/mm^3$, platelet count $\geq 150{,}000/mm^3$, serum bilirubin ≤ 3 mg/dl, alkaline phosphatase no more than twofold elevated, and SGOT no more than threefold elevated.

Study Design and Treatment Plan

HPP, 300 mg orally every 8 hours, was started 24 hours before the first iv bolus injection of 5-FU and was continued for 6 days. The same 5-FU dose was given daily for 5 days unless significant toxicity was observed. The starting dose of 5-FU for all patients was 15–18 mg/kg/day. Treatment was repeated every 4 weeks, the condition of the patient permitting. If no or minimal body weight to a maximum of 24 mg/kg. Dose escalation was carried out both within and between patients. The HPP dose remained constant at 300 mg every 8 hours for 6 days. Measurements of disease, performance status, cbc, platelet count, serum creatinine or BUN, SGOT, bilirubin, and tumor marker were performed prior to each course of treatment. In addition, patients were examined for mucositis, cerebellar signs, and gross visual acuity prior to each treatment course and more frequently if symptoms of toxicity occurred. In reporting the hematologic toxicity, the geometric rather than arithmetic mean was used to reduce the variance. A partial response was defined as a $\geq 50\%$ decrease in the product of perpendicular diameters of measurable lesions. Stable disease was recorded when measurable lesions decreased by $< 50\%$ or increased by $< 25\%$ in the absence of new lesions. Duration of response was considered from the time response was achieved.

RESULTS

Toxicity

Table 1 lists the hematologic values obtained 2 weeks after a course of 5-FU and HPP, as a function of the 5-

TABLE 1.—Hematologic toxicity of 5-FU administered with HPP

Dose level (mg/kg)	No. of patients	No. of courses	Geometric mean at Day 15*		No. of courses/No. evaluable	
			Wbc count (range)	Platelet count (range)	Wbc count $< 2 \times 10^3/mm^3$	Platelet count $< 100 \times 10^3/mm^3$
9	2	4	8.0 (5.3–12)	342 (182–663)	0/4	0/4
15	10	25	7.0 (3.4–12.4)	307 (101–828)	0/23	0/22
18	17	36	4.3 (0.5–10.7)	157 (48–545)	3/36	9/32
21	5	7	3.1 (0.4–5.2)	101 (63–171)	2/7	2/6
24	1	1	1.3	76	1/1	1/1

*Cells $\times 10^3/mm^3$.

FU dose. Two patients could not tolerate the 15-mg/kg dose because of neurotoxicity; one was given three courses and the other was given one course at the 9-mg/kg dose. The Day-15 geometric mean counts fell progressively with increasing 5-FU dose, while there was an increase in the percent of courses in which the leukocyte count fell to $< 2000/mm^3$ or the platelet count fell to $< 100,000/mm^3$. However, myelosuppression was not dose-limiting up to 21 mg/kg, the highest dose for which there were sufficient numbers of courses to make any statement.

Table 2 shows the nonhematologic toxic effects observed during and after each 5-FU and HPP course, as a function of 5-FU dose. When mucositis, nausea, vomiting, and diarrhea occurred they were usually mild and resolved quickly. Nausea, and less often vomiting, tended to occur during the latter days of the 5-FU injections. Mucositis and diarrhea resolved within 2 weeks after the course of 5-FU. These toxic effects were not clearly related to dose, and they were not dose-limiting.

Neurotoxicity occurred with greater frequency than either hematologic or gastrointestinal toxicity and was dose-limiting. Neurotoxic symptoms were usually mild to moderate and consisted of intermittent blurring of vision without a change in visual acuity, decreased sense of balance or "dizziness" that was often associated with an abnormal tandem gait, a positive Romberg test, nystagmus, and abnormal finger-to-nose or heel-to-shin coordination tests. Postural light-headedness, tinnitus, depression, weakness, fatigue, staggering gait (ataxia), shakiness (tremulousness), and slurring of speech were less common symptoms. The onset of neurotoxicity was frequently delayed, and recovery required weeks rather than days. As severity increased, the interval between 5-FU treatments had to be increased from 4 to 6 weeks in most cases. Because of the importance of the dose-limiting neurotoxicity in this study, case histories of two of the most severely affected patients are described in detail.

Case Reports

Case 1.—A 65-year-old man was admitted to the hospital because of increasing urinary and fecal incontinence, inability to stand or walk, and delirium of 1 week's duration. Pulmonary and liver metastases from colon adenocarcinoma had been present for $1^1/_2$ years, but he had received no therapy prior to entering this trial. He had received 5-FU (24 mg/kg, iv bolus) for 5 days, with HPP starting 21 days previously. This was his 12th monthly course of 5-FU and HPP but his first at the 24-mg/kg dose level. He had tolerated treatment at the 21-mg/kg dose level starting 7 weeks prior to admission, without any signs of neurotoxicity. Physical examination at admission showed his vital signs to be normal except for a temperature of 39°C. The liver was 14 cm in span. He was disoriented to time, place, and person but was conscious and responsive to noises and moving objects. His affect was bland; judgment, abstraction, and short-term memory were nil. Coordination and sensory and motor testing were limited by poor patient cooperation. No nystagmus was elicited. Deep tendon reflexes were intact and plantar reflexes equivocal. His hemoglobin was 9 g/100 ml, leukocyte count was 1300/

TABLE 2.—Nonhematologic toxicity of 5-FU administered with HPP

Dose level (mg/kg)	No. of patients	No. of courses	Toxic effect*			
			Mucositis	Diarrhea	Nausea/vomiting	Neurotoxicity
9	2	4	0/4	0/4	0/4	2/4
15	10	24	3/24	3/24	4/24	6/23
18	17	36	10/36	5/36	4/36	17/36
21	5	7	1/7	0/7	1/7	5/7
24	1	1	0/1	1/1	0/1	1/1

*Values = No. of courses with toxic effect/No. evaluable.

mm^3 with 1% neutrophils, and platelet count was 172,000/mm^3. Serum electrolytes, creatinine, sugar, calcium, bilirubin, bromide, vitamin B_{12}, and thyroxine were within normal limits. Lumbar puncture showed normal pressure and clear, colorless cerebrospinal fluid (CSF); no cells were seen. The CSF glucose was 72 mg/dl and the protein was 20 mg/dl. A computerized axial tomographic brain scan showed no masses. An EEG showed a grade I dysrhythmia. In 1 week the neutropenic fever responded to antibiotics and the leukocyte count returned to normal. The patient's mental status improved; he became calm, oriented, and able to remember recent events. He ambulated with minimal assistance and was discharged from the hospital. He was readmitted 2 weeks later because of intermittent delirium, agitation, disorientation, and continued urinary and fecal incontinence. These symptoms persisted without further improvement until death 3 months later. His neurologic deterioration was attributed to his treatment with 5-FU and HPP.

Case 2.—A 62-year-old man was admitted to the hospital because of increasing mental confusion and urinary incontinence for 1 week and chills and fever for 2 days. Liver metastases from colon adenocarcinoma had been diagnosed 2 years previously. Intermittent chemotherapy with 5-FU had been started 11 months previously but was stopped 3 months before the patient entered this trial. His second course of 5-day 5-FU and HPP chemotherapy, administered at a dose of 18 mg/kg/day, had been started 3 weeks before this hospital admission. During examination, his temperature was 39.4°C, pulse was 100/minute, and blood pressure was 100/60. The liver span was 10 cm. He was conscious but moderately confused and agitated. His affect was flat. He could answer yes-no questions appropriately. Within the limits of his cooperation the motor, sensory, cerebellar, and cranial nerve examinations were normal and there were no localizing neurologic signs. The hemoglobin was 11 g/100 ml, leukocyte count was 1500/mm^3 with 2% neutrophils, and platelet count was 76,000/mm^3. Serum electrolytes, glucose, BUN, amylase, and calcium were within normal limits. A lumbar puncture showed normal pressure and clear, colorless CSF with glucose of 77 mg/dl, protein of 42 mg/dl, four rbcs/mm^3, no leukocytes, and negative cytology and bacterial culture. The patient was given iv carbenicillin and gentamicin. Within 2 days he was afebrile. His neutrophil and platelet counts rose to normal. His mental status improved over the 5 days he spent in the hospital. He became oriented, calm, ambulated without assistance, and became capable of self-care. Over the next 3 weeks all symptoms and signs of neurotoxicity remitted.

Response

All 20 patients entered in this trial had measurable disease. One patient with colon carcinoma (case 1) had a partial response of 11 months' duration; he had not received previous chemotherapy. Three other patients with colorectal adenocarcinoma had stable disease lasting 4–7 months. Eight of the patients had received 5-FU chemotherapy prior to entering this 5-FU-HPP study.

DISCUSSION

The maximum tolerated dose of 5-FU is generally accepted to be 12–15 mg/kg given by iv bolus injection for 5 consecutive days, then one-half dose every other day until toxic effects develop (5). Doses as high as 19.2 mg/kg for 5 days have been given to selected individuals (13). In man, as in mice, the coadministration of HPP appears to partially protect the bone marrow and gastrointestinal tract against 5-FU-induced toxicity (3,4). This protective effect allows larger doses of 5-FU to be given when a constant-infusion schedule is used (12). Whether HPP can afford significant protection against single or multiple iv bolus doses of 5-FU is less clear. It was not possible to fully evaluate the effect of HPP on marrow and gut toxicity in this study because of the severity of the neurotoxicity. In another study from this institution (14), we found that when 5-FU was administered concurrently with HPP, or following only 24 hours of HPP pretreatment, no significant protection against myelosuppression was observed. However, it has recently been reported that a 4-day period of HPP pretreatment did afford some protection, and nadir leukocyte counts were significantly higher when the two drugs were given together (15). Thus, with longer periods of pretreatment it may be possible to escalate the dose of 5-FU given on a bolus regimen. Currently available evidence suggests that it is unlikely that HPP will permit more than a doubling of the 5-FU dose.

Neurotoxicity has been observed in all the reported trials of 5-FU in combination with HPP (3,4,12,15) and was dose-limiting in this study. In studies from this institution, the incidence of neurotoxicity was greater when 5-FU was used on an intermittent bolus injection schedule than when it was given as a 5-day constant infusion (4,12,14). This suggests that the very high plasma 5-FU concentrations that are achieved briefly with bolus injections contribute importantly to the development of neurotoxicity. The symptoms and signs of neurotoxicity were similar to those that have been reported previously with high dose rates of 5-FU without HPP (16–18) and were predominantly encephalopathic and cerebellar. The incidence, however, was much higher in this series (31 of 71 courses, 44%), and it increased with dose; however, it should be emphasized that, because of the delayed onset and prolonged duration of the neurotoxic symptoms, it was not always possible to discriminate between the effect of cumulative drug dose and the effect of dose escalation. This is in contrast to

the two previously reported series (3,4) using continuous 24-hour iv infusions of 5-FU with HPP (300 mg every 8 hours orally). The incidence of neurotoxicity in the previous studies was lower: one of 20 (4) and three of 23 (3) patients. The clinical manifestations of neurotoxicity associated with the constant-infusion 5-FU dose schedule were also different, with a predominance of seizures and the absence of cerebellar signs or symptoms.

Neurotoxicity has been observed in other programs in which 5-FU has been combined with a modulating agent. Thymidine by itself causes neurotoxicity only when given at doses $\geq$ 75 g/m^2/day for 5 days (19). By contrast, when 15 g of thymidine was infused over 20 minutes followed 1 hour later by 5-FU at doses of 7.5–10 mg/kg by iv bolus, ten of 13 patients who received more than two courses developed symptoms and/or signs of neurotoxicity (20). Two of 16 patients required hospitalization for "cerebellar dysfunction." Central nervous system symptoms have also been observed with the combination of 5-FU and PALA; mild to moderate lethargy was common (21). The addition of thymidine to 5-FU and PALA produced a 13% incidence of drowsiness, and a 10% incidence of weakness that were not dose-related (22). There is little information on the biochemical basis for the neurotoxicity of 5-FU (23,24). However, the fact that these modulating agents affect the de novo pathway of pyrimidine synthesis and the observation that other drugs inhibiting the same pathway such as 6-azauracil produce a similar pattern of neurotoxicity (25) suggest a central role for pyrimidine metabolism in the neurotoxicity of 5-FU.

REFERENCES

1. Schwartz PM, and Handschumacher RE. Selective antagonism of 5-fluorouracil cytotoxicity by 4-hydroxypyrazolopyrimidine (allopurinol) in vitro. Cancer Res 39:3095–3101, 1979.
2. Schwartz PM, Dunigan JM, Marsh JC, et al. Allopurinol modification of the toxicity and antitumor activity of 5-fluorouracil. Cancer Res 40:1885–1889, 1980.
3. Fox RM, Woods RL, Tattersall MHN, et al. Allopurinol modulation of high dose fluorouracil toxicity. Cancer Treat Rev 6:143–147, 1979.
4. Howell SB, Wung WE, Taetle RE, et al. Modulation of 5-fluorouracil toxicity by allopurinol in man. Cancer 48:1281–1289, 1981.
5. Moertel CG, and Reitemeier RJ. Chemotherapy of gastrointestinal cancer. Surg Clin North Am 47:929–953, 1967.
6. Carrico CR, and Glazer RI. Effect of 5-fluorouracil on the synthesis and transplantation of polyadenylic acid-containing RNA from regenerating liver. Cancer Res 39:3694–3701, 1979.
7. Mandel HG. Incorporation of 5-fluorouracil into RNA and its molecular consequences. Prog Mol Subcell Biol 1:82–135, 1969.
8. Beardmore TD, and Kelley WN. Effects of allopurinol and oxipurinol on pyrimidine biosynthesis in man. *In* Purine Metabolism in Man (Sperling O, DeVries A, and Wyngaarden JB, eds). New York, Plenum Publishing Corp, 1974, pp 609–619.
9. Fox RM, Wood MH, and O'Sullivan WJ. Studies on the coordinate activity and liability of orotidylate phosphoribosyltransferase and decarboxylase in human erythrocytes and the effects of allopurinol administration. J Clin Invest 50:1050–1060, 1971.
10. Reyes P, and Guganig ME. Studies on a pyrimidine phosphoribosyltransferase from murine leukemia P1534J. J Biol Chem 250:5097–5108, 1975.
11. Nahas A, Savlov ED, and Hall TC. Phosphoribosyl transferase in colon tumor and normal mucosa as an aid in adjuvant chemotherapy with 5-fluorouracil (NSC-19893). Cancer Chemother Rep 58:909–912, 1974.
12. Kroener JF, Saleh F, and Howell SB. 5-FU and allopurinol: toxicity modulation and phase II results in colon cancer. Cancer Treat Rep 66:1133–1137, 1982.
13. Siber GR, Mayer RJ, and Levin MJ. Increased gastrointestinal absorption of macromolecules after 5-fluorouracil therapy. Cancer Res 40:3430–3436, 1980.
14. Howell SB, Pfeifle CE, and Wung WE. Effect of allopurinol on the toxicity of 5-fluorouracil administered by intermittent bolus injection. Lancet. In press.
15. Woolley PV, Ayoob MJ, Smith FP, et al. A controlled trial of the effect of allopurinol on the toxicity of a single bolus dose of 5-fluorouracil. Proc Am Assoc Cancer Res and ASCO 1:20, 1982.
16. Moertel CG, Reitemeier RJ, Bolton CF, et al. Cerebellar ataxia associated with fluorinated pyrimidine therapy. Cancer Chemother Rep 41:15–18, 1964.
17. Riehl J-L, and Brown WJ. Acute cerebellar syndrome secondary to 5-fluorouracil therapy. Neurology 14:961–967, 1964.
18. Bixenman WW, Nicholls JVV, and Warwick OH. Oculomotor disturbances associated with 5-fluorouracil chemotherapy. Am J Opthalmol 83:789–793, 1977.
19. Chiuten DF, Wiernik PH, Zaharko DS, et al. Phase I–II trial and clinical pharmacology of continuously infused high dose thymidine. Proc Am Assoc Cancer Res and ASCO 20:305, 1979.
20. Cheng E, Woodcock T, Young C, et al. Enhanced neurotoxicity of 5-fluorouracil by thymidine. Proc Am Assoc Cancer Res and ASCO 21:350, 1980.
21. Casper ES, Vale K, Sykes MP, et al. Clinical and pharmacologic studies on biochemical modulation of fluorouracil with phosphonacetyl-L-aspartate (PALA). Proc Am Assoc Cancer Res and ASCO 22:165, 1981.
22. Chiuten DF, Benvenuto J, Ho DH, et al. Phase I clinical and pharmacokinetic study of thymidine, 5-fluorouracil and *N*-(phosphonacetyl)-L-aspartic acid (PALA) in combination. Proc Am Assoc Cancer Res and ASCO 22:358, 1981.
23. Koenig H, and Patel A. Biochemical basis of the acute cerebellar syndrome in 5-fluorouracil chemotherapy. Trans Am Neurol Assoc 94:290–292, 1969.
24. ———. The acute cerebellar syndrome in 5-fluorouracil chemotherapy: a manifestation of fluoroacetate intoxication. Neurology 20:416, 1970.
25. Handschumacher RE, and Pasternak CA. Inhibition of orotidylic acid decarboxylase, a primary site of carcinostasis by 6-azauracil. Biochim Biophys Acta 30:451–452, 1958.

Phase II Study of Vindesine in Patients With Advanced Breast Cancer[1,2]

Brian K. Walker,[3,*] Peter C. Raich,[3] Joseph Fontana,[3] V. P. Subramanian,[3,4] John S. Rogers, II,[3] James A. Knost,[3,5] and Beth Denning[3]

Vindesine, a new vinca alkaloid, was administered to 20 patients with advanced refractory breast cancer in a phase II trial. The drug was given at a dose of 3 mg/m² by iv bolus each week for 6 consecutive weeks, and responding patients were maintained on a dose of 4 mg/m² every 2 weeks. Nineteen patients were evaluable for disease response; partial remissions were obtained in five patients, for a response rate of 26%. Leukopenia was the major dose-limiting toxic reaction, but most patients were able to tolerate this schedule without difficulty. Neurotoxicity was mild and did not require dose reduction. [Cancer Treat Rep 66:1729-1732, 1982]

Vindesine is a new semisynthetic vinca alkaloid with structural similarity to vinblastine. Preclinical studies in animal tumor systems have demonstrated a spectrum of antitumor activity wider than that of vinblastine and very similar to that of vincristine. However, in the preclinical investigations, vindesine has produced fewer neurotoxic reactions than vincristine and less myelosuppression than vinblastine (1).

Initial phase I–II clinical studies have examined the efficacy and toxicity of vindesine administered by iv bolus at 1–2 week intervals. The major dose-limiting toxic effect in most phase I trials has been leukopenia. Mild to moderate neurotoxicity was frequently noted, but seldom required dose reduction. Patients receiving the drug on a weekly schedule have tolerated a dose of 2–4 mg/m² without excessive side effects (2).

The results of early phase II trials indicate that vindesine has significant antitumor activity in breast cancer. Four published phase II studies have demonstrated a response rate of 18%–29% when the drug is given on a weekly iv schedule (3–6). However, not all investigators have observed this degree of activity, with two trials producing a response rate of only 0%–4% (7,8).

This report presents the results of a phase II trial of vindesine in advanced breast cancer using a dose and schedule similar to prior studies. We are able to confirm the activity of vindesine in refractory breast cancer.

METHODS

Twenty patients with histologically proven breast cancer and recurrent disease were entered in this study. All patients had failed prior combination chemotherapy and those treated with hormonal therapy had demonstrated progressive disease after withdrawal of the hormonal agent. At least one site of measurable/evaluable disease was required. Other criteria for entry in this protocol included age < 70 years, Eastern Cooperative Oncology Group (ECOG) performance status ≤ 2, normal hepatic and renal function, a leukocyte count > 3000/mm³, and a platelet count > 50,000/mm³. All patients were required to sign an informed consent statement and be geographically accessible to followup.

Vindesine was supplied by Eli Lilly and Co. The treatment schedule consisted of vindesine (3 mg/m²), given by iv bolus for 6 consecutive weeks as tolerated by blood cell counts and toxic reactions. Patients with evidence of disease response or stable disease at the completion of the initial 6-week induction phase were then maintained on a dose of 4 mg/m² every 2 weeks until evidence of disease progression. The following sliding scale was used for dose adjustment based on a cbc performed on the day of treatment: for wbc count > 4000/mm³ and platelet count > 100,000/mm³, 100% dose; for wbc count of 3000–4000/mm³ or platelet count of 50,000–100,000/mm³, 50% dose; for wbc count < 3000/mm³ or platelet count < 50,000/mm³, therapy was delayed until the blood cell counts improved.

All patients were seen on a weekly basis and evaluated for both drug toxicity and disease response. Preliminary studies prior to treatment included a complete history and physical examination, cbc with differential, platelet count, urinalysis, serum chemistry profile, chest X-ray and X-rays of indicator lesions, bone scan, liver scan, and biopsy of recurrent disease as appropriate.

[1]Received Apr 5, 1982; accepted June 16, 1982.

[2]Presented in part at the 12th International Congress of Chemotherapy, Florence, Italy, July 19–24, 1981.

[3]Department of Medicine, West Virginia University School of Medicine, Morgantown.

[4]*Present address:* Department of Medicine, Olin E. Teague Veterans Administration Hospital, Temple, TX.

[5]*Present address:* Biological Response Modifiers Program, National Cancer Institute, Frederick Cancer Research Center, MD.

**Reprint requests to:* Brian K. Walker, MD, Department of Medicine, West Virginia University School of Medicine, Morgantown, WV 26506.

Followup evaluation during therapy included a weekly cbc and platelet count as well as assessment of disease response and drug toxicity. Studies performed at 4-week intervals included a serum chemistry profile, chest X-ray and X-rays of indicator lesions, and liver scan when indicated. Repeat bone scans were performed at 12-week intervals as appropriate.

The criteria for evaluating disease response were defined as follows: *complete response (CR)*— the complete regression of all measurable disease, *partial response (PR)*—a decrease of at least 50% of the tumor area in all involved sites for a minimum of 4 weeks, and *progressive disease*—a 25% increase in the area of any existing lesion or the appearance of a new lesion. Stable disease status was assigned to patients not meeting these criteria. Response duration was calculated from the beginning of chemotherapy until evidence of progressive disease.

RESULTS

Twenty patients were entered in this study and all had received prior chemotherapy. Nine patients had demonstrated progressive disease while receiving prior vincristine therapy. No patient received prior therapy with vinblastine. Ten of the 20 patients had received previous hormonal therapy and demonstrated subsequent progressive disease. Details of the patient characteristics are listed in table 1.

Only one patient completed < 4 weeks of therapy due to an early death (1 week) and therefore 19 of the 20 patients were evaluable for disease response and drug toxicity. The initial 6-week cycle of vindesine therapy was well-tolerated, with 18 of the 19 evaluable patients completing this phase of the treatment. One patient received only four weekly treatments due to early progressive disease. After the initial 6-week cycle, all patients with disease response or stable disease were treated every 2 weeks with a dose escalation to 4 mg/m^2. The median number of treatments administered was ten (range, four to 25). The number of treatments administered with a dose reduction due to toxicity was minimal (median, two; range, none to 11).

There were no CRs, but five patients achieved a PR, for a total response rate of 26%. Sites of disease response included soft tissue (two), osseous (two), and lung (one). The duration of disease response ranged from 14 to 56 weeks. Two of the five PRs were in patients who had failed prior vincristine therapy. Details of the responding patients are listed in table 2.

The major toxic reaction encountered in this study was leukopenia. Fifteen of the 19 evaluable patients had at least one episode of leukopenia (wbc count < 4000/mm^3). However, only one patient had severe leukopenia with a wbc count nadir of 1600/mm^3, and no patient developed infection or sepsis related to the myelosuppression. Mild to moderate anemia developed in 89% of the patients but did not require dose reduction. Only one patient developed a platelet count of < 100,000/mm^3, and no bleeding complications were noted. An interesting finding was that 15 patients manifested an

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Total in study	20
Menopausal status	
Premenopausal	2
Postmenopausal (natural)	15
Postmenopausal (surgical)	3
ECOG performance status	
0	5
1	9
2	6
Disease-free interval (yrs)	
< 1	5
1–3	11
3–5	2
> 5	2
Sites of disease	
Osseous	16
Soft tissue/nodes	14
Lung	7
Liver	2
Previous chemotherapy	20
Previous hormonal therapy	10
Previous vinca alkaloids	9
Age in yrs	
Median: 61	
Range: 49–69	

TABLE 2.—Responding patients

Patient No.	Response	Site of disease	Duration of response (wks)	Prior vinca alkaloids
1	PR	Lung	18	Vincristine
2	PR	Soft tissue	36	No
3	PR	Soft tissue	14	No
4	PR	Osseous	16	Vincristine
5	PR	Osseous	56	No

TABLE 3.—Vindesine toxicity

Toxic effect	No. of patients (%)
Leukocyte count (cells/mm³)	
> 4000	4 (21)
3000–4000	4 (21)
2000–3000	10 (53)
1000–2000	1 (5)
Hemoglobin (g/dl)	
> 11	9 (47)
10–11	8 (42)
< 10	2 (11)
Platelet count (cells/mm³)	
> 100,000	18 (95)
< 100,000	1 (5)
Increase of > 100,000	15 (79)
Neurotoxic effects	
Paresthesias	15 (79)
Weakness	10 (53)
Constipation	6 (32)
Miscellaneous	
Alopecia	4 (21)
Nausea/vomiting	4 (21)
Skin rash	1 (5)
Fever	1 (5)

increase in platelet count of at least 100,000/mm³ above baseline during vindesine therapy.

The neurotoxic effects encountered in this study were mild and did not require dose reduction in any patient. Peripheral neuropathy (79%) and generalized weakness (53%) were common, with constipation noted by 32% of the patients. Miscellaneous side effects included alopecia (21%), nausea and vomiting (21%), and skin rash and fever occurring in one patient each. Details of vindesine toxicity are listed in table 3.

DISCUSSION

The response rate of 26% observed in this study of patients with refractory breast carcinoma compares favorably to other phase II trials of vindesine at similar dose schedules. The dose selected for this trial was based on reports from earlier studies which suggested that the drug was most effective when administered on a weekly schedule, and that doses > 3 mg/m² produced myelosuppression and neurotoxicity requiring delay in the treatment program (2,9). The dose selected for this trial was well-tolerated, with 18 of the 19 evaluable patients completing the initial 6-week induction phase. Furthermore, the median number of treatments administered was ten, with dose reduction due to toxicity required in only 31% of the treatments. Interruption of the planned treatment schedule due to severe myelosuppression was seldom necessary.

The five PRs observed in this study were in heavily pretreated patients. All responding patients had received both prior chemotherapy and hormonal therapy. In addition, two of the five patients had demonstrated progressive disease on previous vincristine therapy, supporting the contention that vindesine is non-cross-resistant with other vinca alkaloids (4,5).

Phase II trials of vindesine in breast carcinoma, using a weekly iv bolus schedule, have been reported from seven institutions, including our own. These studies are summarized in table 4. A total of 136 patients were evaluable for disease response, with CRs in three and PRs in 23, for a total response rate of 19.1%. The two trials that documented a poor response rate involved larger doses with subsequent toxicity and delay in the treatment schedule. The five phase II trials demonstrating an improved response rate had the common feature of a lower dose (3 mg/m²/week) with less schedule interruption. The antitumor activity of vindesine in breast carcinoma is apparently improved with the lower dose administered on a regular weekly schedule.

The major toxic effect encountered in this phase II trial was leukopenia, with 15 of 19 patients (79%) experiencing at least one leukocyte count nadir < 4000/mm³. However, only one patient had a leukocyte count < 2000/mm³, and no episodes of infection or sepsis were encountered. Mild to moderate anemia was observed in ten of 19 patients (53%), but dose reductions were not required. Thrombocytopenia was noted in only one patient, and in most cases an increase in platelet count was observed. Fifteen of the 19 evaluable patients (79%) demonstrated an increase in platelet count of at least 100,000/mm³ above baseline during the course of vin-

TABLE 4.—Phase II trials of vindesine in breast cancer (weekly iv bolus schedule)

Investigator (ref No.)	Dose (mg/m²/wk)	No. of evaluable patients	No. of CRs	No. of PRs	Response rate
Smith et al (3)	3	21	2	4	6/21 (29%)
Cobleigh et al (4)	3	21	0	6	6/21 (29%)
Robins et al (5)	3	31	1	5	6/31 (19%)
Miller et al (6)	3	11	0	2	2/11 (18%)
Ohnuma et al (7)	2–5	8	0	0	0/8 (0%)
Currie et al (8)	3–4	25	0	1	1/25 (4%)
Walker et al (this report)	3	19	0	5	5/19 (26%)
Total		136	3	23	26/136 (19.1%)

desine therapy. This relative platelet-sparing effect for vindesine has been noted by other investigators (3,10).

Neurotoxicity was relatively mild and did not require dose reduction in any patient. Paresthesias and generalized weakness were reported by most patients, and approximately one third noted some constipation. Other miscellaneous side effects were less common and included alopecia, nausea, vomiting, skin rash, and fever.

In conclusion, vindesine is an active agent in advanced breast cancer, with a response rate of 26% observed in this phase II trial. The dose-limiting toxic reaction was mild to moderate leukopenia. A review of the published phase II results for vindesine in breast cancer suggests that the agent is effective when administered on a regular weekly schedule at a relatively low dose.

REFERENCES

1. Dyke RW, and Nelson RL. Phase I anti-cancer agents: vindesine (desacetyl vinblastine amide sulfate). Cancer Treat Rev 4:135–142, 1977.
2. Currie VE, Wong PP, Krakoff IH, et al. Phase I trial of vindesine in patients with advanced breast cancer. Cancer Treat Rep 62:1333–1336, 1978.
3. Smith IE, Hedley DW, Powles TJ, et al. Vindesine: phase II study in the treatment of breast carcinoma, malignant melanoma, and other tumors. Cancer Treat Rep 62:1427–1433, 1978.
4. Cobleigh MA, Williams SD, and Einhorn LH. Phase II study of vindesine in patients with metastatic breast cancer. Cancer Treat Rep 65:659–663, 1981.
5. Robins HI, Tormey DC, Skelley MG, et al. Vindesine: a phase II trial in advanced breast cancer patients. Cancer Clin Trials 4:371–375, 1981
6. Miller TP, Jones SE, and Chester A. Phase II trial of vindesine in the treatment of lymphomas, breast cancer, and other solid tumors. Cancer Treat Rep 64:1001–1003, 1980.
7. Ohnuma T, Greenspan EM, and Holland JF. Initial clinical study with vindesine: tolerance to weekly iv bolus and 24-hour infusion. Cancer Treat Rep 64:25–30, 1980.
8. Currie VE, Camacho F, Wittes R, et al. Phase II evaluation of vindesine in patients with advanced breast cancer. Cancer Treat Rep 64:693–695, 1980.
9. Valdivieso M, Richman S, Burgess AM, et al. Initial clinical studies of vindesine. Cancer Treat Rep 65:873–875, 1981.
10. Gralla RJ, Tan CTC, and Young CW. Vindesine: a review of phase-II trials. Cancer Chemother Pharmacol 2:271–274, 1979.

Pharmacokinetics of Methotrexate and 7-Hydroxymethotrexate Following Infusions of High-Dose Methotrexate[1,2]

Henning Breithaupt[3,*] and Eberhard Küenzlen[4,5]

The pharmacokinetics of methotrexate (MXT) and 7-hydroxymethotrexate (7-OH-MTX) has been studied in seven patients treated for osteosarcoma with up to 27 cycles of MTX at doses of 140–350 mg/kg of bc .y weight. The distribution volume of MTX was 0.186 ± 0.062 liter/kg. Peak plasma levels ranged from 540 to 1700 μM for MTX and from 12 to 560 μ M for 7-OH-MTX. The MTX metabolite 2,4-diamino-N^{10}-methylpteroic acid was occasionally detectable in plasma and urine at a level of 10^{-7}M. Plasma disappearance of MTX was biphasic, with a terminal half-life of 2.1 ± 0.6 hours (mean ± SE). Plasma decay of 7-OH-MTX was mainly monoexponential, with a half-life of 23.8 ± 15.2 hours. The renal clearance of MTX (0.0623 ± 0.0232 liter/kg/hour) accounted for about 84% of the total clearance of MTX (0.0742 ± 0.0288 liter/kg/hour). In urine, 70%–94% of the dose was recovered as MTX and 0.4%–11% as 7-OH-MTX. The renal clearance of 7-OH-MTX was in the range of 0.0173 ± 0.0149 liter/kg/hour. The pharmacokinetics of MTX and 7-OH-MTX was found to be highly reproducible intraindividually. The kinetics of MTX and 7-OH-MTX was influenced by orally coadministered activated charcoal, presumably by inhibition of enteral reabsorption of MTX and 7-OH-MTX. [Cancer Treat Rep 66:1733–1741, 1982]

High-dose methotrexate (MTX) (> 1 g/m²) with leucovorin rescue is widely used as adjuvant therapy for osteosarcoma (1,2). Drug-related deaths secondary to high-dose MTX and citrovorum factor administration have been reported to occur in 6% of patients, mainly because of severe myelosuppression or renal failure (3). The safety of high-dose MTX was increased by hydration and alkalinization (4,5). Routine monitoring of plasma MTX concentrations is recommended for early detection of patients at a high risk of toxicity, who will require increased doses and prolonged courses of leucovorin (5,6).

A specific and sensitive high-pressure liquid chromatography (HPLC) method has been developed (7) for routine monitoring and pharmacokinetic studies. This paper presents the characteristics of plasma disappearance and urinary excretion of MTX and its main metabolite 7-hydroxymethotrexate (7-OH-MTX) in seven patients treated for osteosarcoma with high-dose MTX.

MATERIALS AND METHODS

Therapeutic Regimen

Seven patients with osteosarcoma (16–48 years of age) were treated with high-dose MTX between December 1977 and July 1981. The patients received up to 27 drug cycles of high-dose MTX every 3–4 weeks. A total of 122 cycles were given.

MTX doses of 140–350 mg/kg of body weight were administered by continuous iv infusion over 6 hours followed by citrovorum factor rescue. Seven doses of citrovorum factor (15 mg each) were injected iv every 6 hours, beginning 3 hours after completion of the MTX infusion. Iv fluids (5% glucose) were administered at a rate of 3500 ml/24 hours with 70 mEq of $NaHCO_3$/liter, starting 3 hours prior to the MTX infusion and continuing for 40 hours thereafter to ensure a large volume of alkaline urine.

Determination of MTX and Its Metabolites

Blood samples (0.5–1.0 ml) were collected at specified time intervals into lheparinized tubes and plasma was chromatographed without further pre-cleanup procedure. Plasma samples not immediately analyzed were stored frozen at −16°C.

Urine samples were diluted 1:20 up to 1:2000 in distilled water and then samples of 50 μl were injected into the chromatographic system. Plasma and urine concentrations of MTX, 7-OH-MTX, and 2,4-diamino-

[1]Received Nov 30, 1981; revised Mar 9, 1982; accepted May 25, 1982.

[2]Supported by the Deutsche Forschungsgemeinschaft, Bonn-Bad Godesberg, FRG, grants Br 587/5 and Br 587/7.

[3]Medizinische Klinik der Justus Liebig-Universität, Giessen, Federal Republic of Germany.

[4]Institut für Transfusionsmedizin und Klinische Immunologie im Zentrum für Klinische Chemie, Klinische Immunologie und Humangenetik, Giessen, Federal Republic of Germany. E. Küenzlen will present this work as part of his thesis to the Fachbereich Medizin der Justus Liebig-Universität.

[5]We thank Prof. Dr. H. M. von Hattingberg for evaluation of pharmacokinetic data, and Dr. H. Pralle for referral of patients and helpful discussions.

Reprint requests to: Dr. H Breithaupt, Medizinische Klinik der Justus Liebig-Universität, Klinikstrasse 36, D-6300 Giessen, Federal Republic of Germany.

N^{10}-methylpteroic acid (APA) were determined by HPLC using a Perisorb RP2-precolumn (3 × 0.4 cm, inside diameter) connected to a Spherisorb 5-μ ODS analytic column (15 × 0.4 cm) as stationary phase, and a ternary solvent mixture of citrate-phosphate (0.05 M, pH 3.2): methanol:tetrahydrofurane (80:16:4, by vol) as eluent. The pressure was maintained at 170 bar resulting in a flow rate of 2.0 ml/minute. Detection was set at 303 nm using a variable-wavelength photometer (Uvikon 720 LC, Kontron). MTX was eluted from the column at 3 minutes, 7-OH-MTX at 5.5 minutes, and APA at 8 minutes. The sensitivity of the assay system was 10^{-7} M for each substance, with a coefficient of variation < 7%. At 4×10^{-8} M the within-run coefficient of variation was 14.7% for MTX and 20.3% for 7-OH-MTX. Further details are published elsewhere (7).

Pharmacokinetic Calculations

Half-lives were calculated from plasma data by nonlinear curve fitting, based on the Gauss-Newton method with dynamic Hartley's modification (8). The computations were done by rotative iterative procedures on a Hewlett-Packard 9825 calculator. The total clearance (Cl_{total}) was obtained by dividing the total dose by the area under the curve (AUC) between the start of infusion and infinity. AUC was computed from the experimental data by parabolic interpolation and was extrapolated to infinity using the terminal slope. Extrapolation contributed < 1% to the MTX area and usually < 10% to the AUC of 7-OH-MTX. The total volume of distribution (V_{ss}) was obtained as the product of Cl_{total} and the mean transit time (T). T is computed as the area between the curve of cumulative disposition and its asymptote divided by this asymptote (8). The mean time of constant infusion, eg, 0.5 × infusion time, is subtracted from the mean system time to obtain the mean transit (or residence) time T for the drug in the body.

The fraction of MTX converted into 7-OH-MTX was not determined in this study. Volume terms are therefore not representative for the metabolite. From urinary data the renal clearances (Cl_{renal}) of MTX and 7-OH-MTX were evaluated. The values of V_{ss}, half-life, Cl_{total}, and Cl_{renal} are presented in table 1.

RESULTS

A typical time course of plasma concentrations of MTX and 7-OH-MTX during and after an infusion of high-dose MTX is shown in figure 1. Computer calculation of the plasma data indicates that elimination of MTX

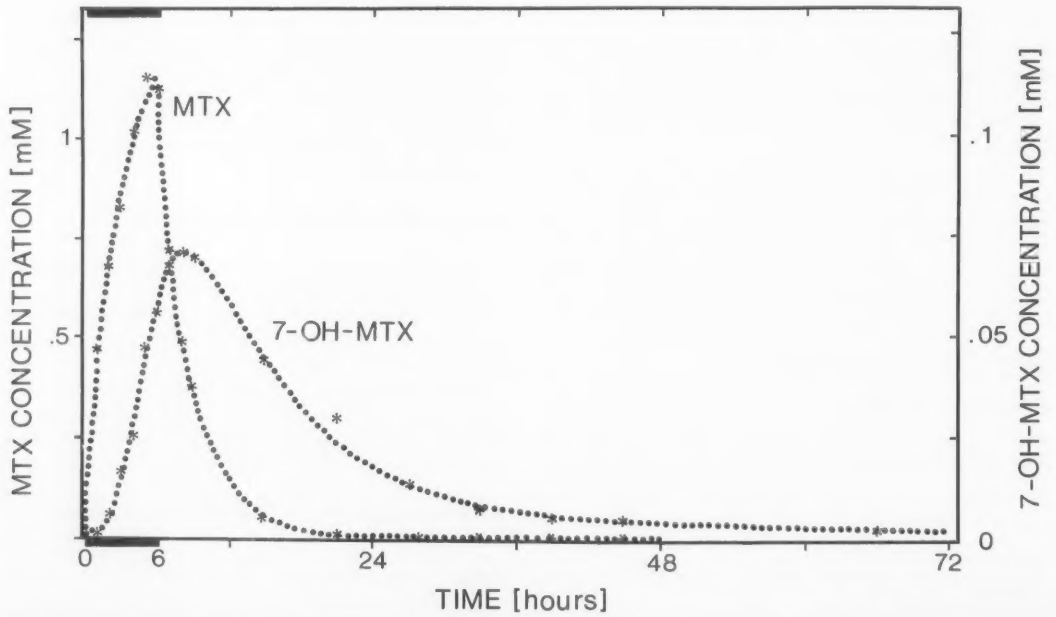


FIGURE 1.—Plasma kinetics of MTX and 7-OH-MTX during and after a continuous 6-hr infusion of 15 g of MTX (211 mg/kg; patient 4). The asterisks (*) represent observed values, whereas the points (●) were obtained by a computerized curve fitting.

TABLE 1.—Kinetic data of MTX and 7-OH-MTX following 6-hr infusions of high-dose MTX

		Kinetic data of MTX					Kinetic data of 7-OH-MTX		
Patient No.	Dose (mg/kg)	C_{max}* (μM)	V_{ss} (liter/kg)	β-half-life (hrs)	Cl_{total} (liter/kg/hr)	Cl_{renal} (liter/kg/hr)	C_{max} (μM)*	Half-life (hrs)	Cl_{renal} (liter/kg/hr)
1	204	1100	0.177	1.20	0.0778	0.0617	230	7.56	0.0137
2	200	580	0.179	2.75	0.1140	0.0769	100	11.0	0.0503
3	152	1100	0.142	2.13	0.0493	0.0376	290	22.2	0.0094
4	141	790	0.158	1.88	0.0547	0.0506	48	81.4	0.0155
	211	1100	0.177	2.29	0.0573	0.0534	71	40.8	0.0065
	282	1624	0.161	2.89	0.0467	0.0438	120	42.8	0.0118
5	137	620	0.191	2.11	0.0746	0.0677	65	37.5	0.0146
	205	1000	0.188	2.55	0.0599	0.0555	130	33.9	0.0096
	274	1700	0.156	3.51	0.0386	0.0372	110	31.6	0.0082
6	233	544	0.426	1.71	0.1660	0.1540	12	9.58	0.0269
	349	1124	0.269	2.19	0.0882	0.0839	118	11.0	0.0131
7	145	910	0.142	1.45	0.0569	0.0524	140	33.0	0.0077
	217†	1200	0.217	15.08	0.0392	0.0355	560	34.4	0.0050
Mean‡			0.186	2.08	0.0742	0.0623		23.8	0.0173
SE			0.062	0.58	0.0288	0.0232		15.2	0.0149

*Maximal concentration in plasma.
†Drug cycle followed by severe mucositis.
‡Mean of each patient was used for calculation (with exception of the toxic drug cycle of patient 7).

follows a two-compartment model, and elimination of 7-OH-MTX occurs mainly monoexponentially.

Plasma kinetics of MTX and 7-OH-MTX in a patient who received 6-hour infusions of 10, 15, and 5 × 20 *g* of MTX are shown in figure 2. The correlation between the peak plasma levels of MTX and 7-OH-MTX and the MTX dose applied was good. Records of five consecutive infusions of 20 *g* of MTX (282 mg/kg) revealed very similar plasma kinetics of MTX and 7-OH-MTX. The plasma values at indicated time intervals varied within a ± 1 SE range < 11%.

A scattergram of all MTX plasma values obtained from 122 high-dose infusions (fig 3a) shows that by 24–72 hours after the end of infusion most MTX plasma levels were below the toxic borderline reported to be 10^{-5} M at 24 hours, 10^{-6} M at 48 hours, and 10^{-7} M at 72 hours (5,9–14). Only one drug cycle was followed by plasma levels of MTX that were above this limit. Clinically a severe stomatitis occurred in this patient which subsided after 2 weeks. All other 121 high-dose MTX infusions had "nontoxic" MTX plasma kinetics, and there was no occurrence of severe toxic reactions except in one patient (No. 5) who repeatedly had serious hepatotoxic effects.

The plasma concentrations of 7-OH-MTX during and after 122 high-dose MTX infusions are shown in figure 3b.

The peak plasma levels of MTX were in the range of 540–1700 μM at the end of high-dose MTX infusion (table 1). Three hours after the end of infusion there were maximal plasma concentrations of 7-OH-MTX at 12–560 μM. Due to its slower disappearance from plasma, 7-OH-MTX exceeded the plasma concentrations of MTX at 3–10 hours following infusion of high-dose MTX.

When MTX plasma levels were in the range of 10^{-7} M, 7-OH-MTX was found to be 17–140 times higher than MTX.

The overall kinetic data obtained from seven patients treated with repeated drug cycles are listed in table 1. The total volume of distribution of MTX was 0.186 ± 0.062 liter/kg. The terminal β-half-life values of MTX were in the range of 2.08 ± 0.58 hours (mean ± SE), being about four times higher than the initial half-life values (0.58 ± 0.15 hours). Upon repeated treatment with high-dose MTX the intra-individual variation of the terminal half-life of MTX was < 7%, whereas its variation among patients was 28%. The mean time of MTX was 2.67 ± 0.61 hours, and the total clearance was found to be 0.072 ± 0.0288 liter/kg/hour. The renal clearance of MTX as calculated from urinary data was 0.0623 ± 0.0232 liter/kg/hour, indicating a metabolic (or nonrenal) contribution of about 16% to the total clearance of MTX.

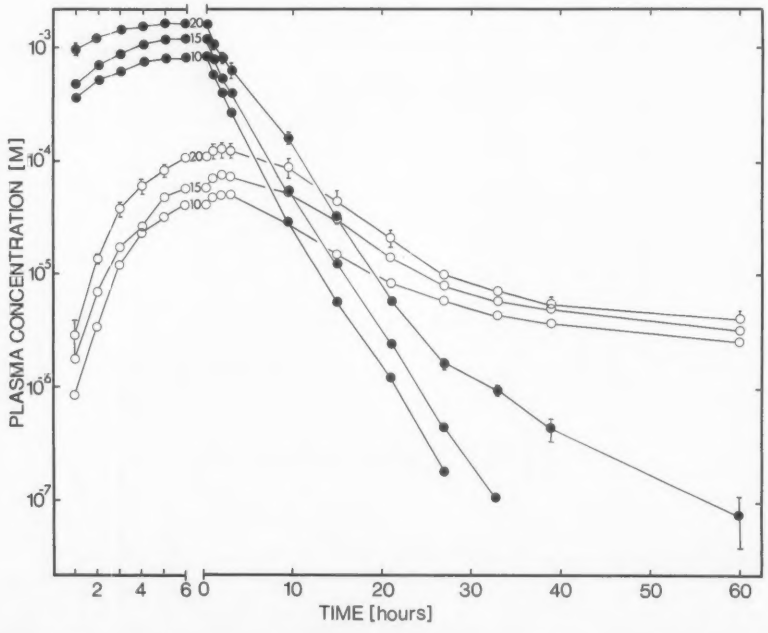


FIGURE 2.—Plasma concentrations of MTX (●) and 7-OH-MTX (○) in 1 patient (No. 4) treated with repeated 6-hr infusions of high-dose MTX (1 × 10 g ≙ 141 mg/kg; 1 × 15 g ≙ 211 mg/kg; 5 × 20 g ≙ 282 mg/kg). 20-g infusions were applied at 4-wk intervals, and their plasma values are shown as mean ± 1 SE.

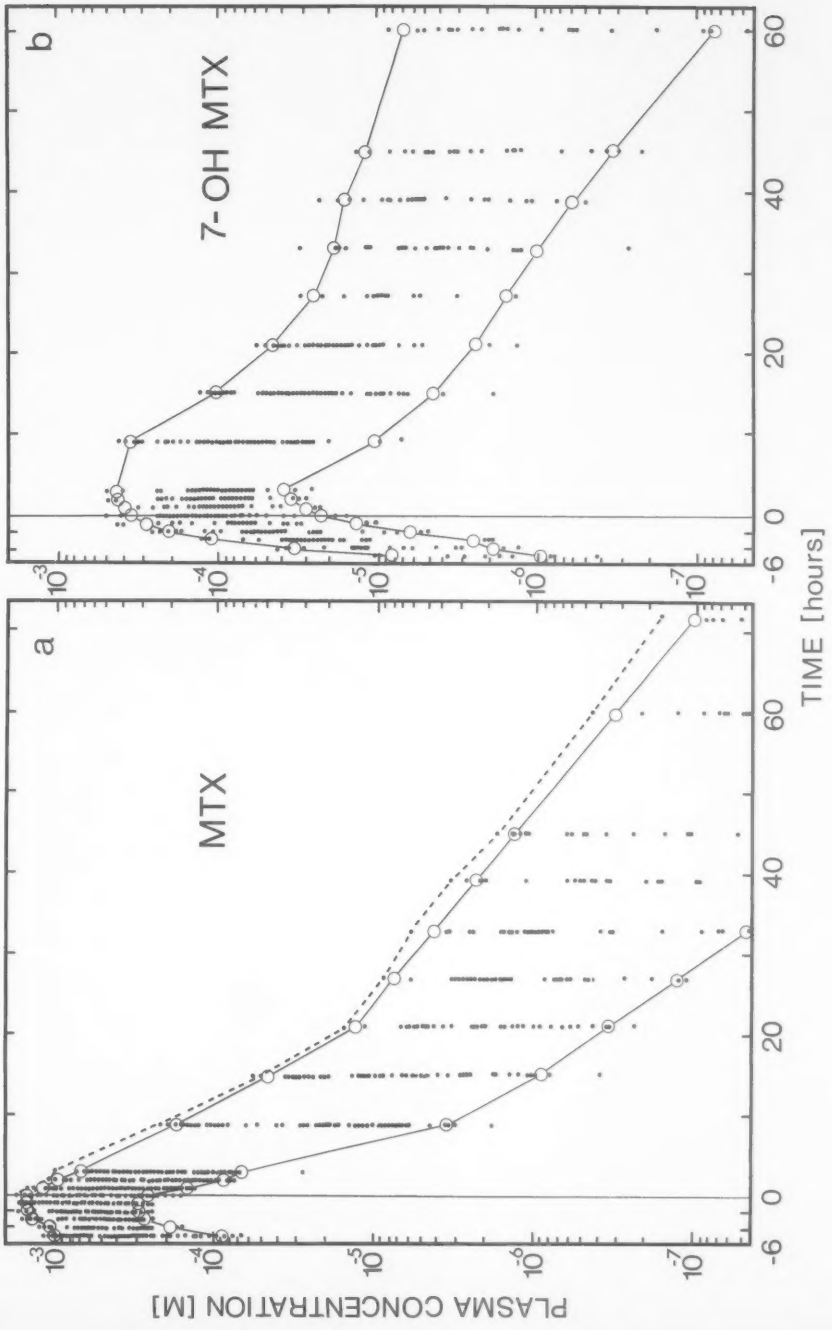


FIGURE 3.—Scattergrams of plasma concentrations of MTX (a) and 7-OH-MTX (b) following 122 6-hr MTX infusions of 140–350 mg/kg. Dotted line shows the MTX plasma kinetics of a drug cycle which was followed by severe mucositis (patient 7). Solid lines represent the 5 and 95 percentiles.

In contrast to the elimination of MTX, the plasma disappearance of 7-OH-MTX was mainly monoexponential, with half-life values of 23.8 ± 15.2 hours (mean ± SE). The renal clearance of 7-OH-MTX was 0.0173 ± 0.0149 liter/kg/hour.

The cumulative urinary excretion of MTX and 7-OH-MTX as monitored over a period of 120 hours is presented in figure 4. About 50% of the dose of MTX was excreted within the first 12 hours after the start of infusion, and about 80% was recovered within the first day. As shown in table 2, recovery of MTX ranged from 70.2% to 94.0% (mean, 85.4), whereas 0.4%–11.1% (mean, 3.8) was excreted as 7-OH-MTX. The recovery of MTX and 7-OH-MTX was quite reproducible intra-individually on repeated application of high-dose MTX.

The MTX metabolite APA was found in the urine in small amounts of up to 0.01% of the applied dose. In some plasma samples APA was demonstrable up to a concentration of 10^{-7} M.

Some evidence for biliary secretion and enterohepatic recirculation of MTX and 7-OH-MTX is provided by the following data: First, a considerable amount of MTX (480 mg) and 7-OH-MTX (63 mg) was found in the gastric juice vomited at the end of a high-dose MTX infusion. The gastric juice was contaminated by some bilirubin glucuronide (1.9 mg/100 ml). Second, the plasma disappearance of MTX and 7-OH-MTX was influenced by coadministration of activated charcoal, which was given orally for 3 days beginning 12 hours before the start of the high-dose MTX infusions (fig 5). Following daily administration of 10 g of activated charcoal, the plasma half-life of MTX was 84% and the half-life of 7-OH-MTX was 44% of the controls, whereas the total clearance of MTX increased by 112%. During the drug cycles with coadministered charcoal the urinary recovery decreased by 11% with respect to MTX and by 71% for 7-OH-MTX.

DISCUSSION

Previous data on the kinetics of high-dose MTX were based on assays that could not differentiate the metabolites (5,13,15–29), or were influenced by immunologic cross-reactions (14,30). The cross-reactivity of 7-OH-MTX known to be in the range of 1%–10% (31–34) is relevant for the determination of MTX in the final phase of disappearance from plasma at 48–72 hours, since 7-OH-MTX levels were shown to be up to 140 times higher than those of MTX. The cross-reactivity of the MTX metabolite APA, which was occasionally detected in plasma and urine (7), has been reported to be 100% in the immunologic assays (31–35). Therefore, the accuracy of the test methods used in previous kinetic studies remains questionable, in particular when applied to plasma samples collected in the terminal phase of MTX elimination.

The discrepancy between the terminal half-life value of MTX in this study and the half-life values of 5.6–16 hours reported previously (19,21,23,27,29) for the post-infusion period from 15 to 60 hours may be caused by

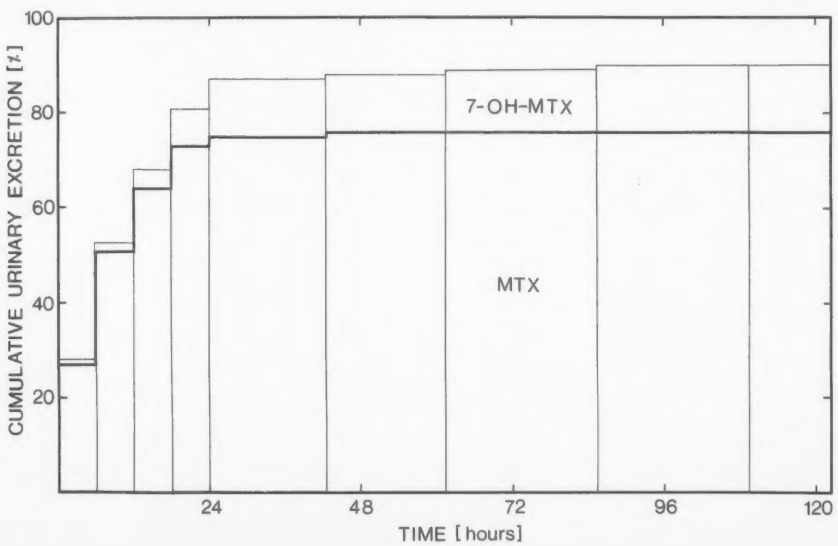


FIGURE 4.—Cumulative excretion of MTX and 7-OH-MTX in urine. 10 g of MTX (152 mg/kg) was administered to the patient (No. 3) by a continuous 6-hr infusion.

TABLE 2.—Urinary recovery following treatment with high-dose MTX.

Patient No.	Dose (g)	Drug cycles	Recovery of MTX (%)		Recovery of 7-OH-MTX (%)	
			Mean	SE	Mean	SE
1	10	6	78.7	12.5	3.9	2.2
2	10	9	70.2	16.9	. 3.5	2.3
3	10	12	75.7	7.5	11.1	3.8
4	10–20	12	93.2	7.8	2.7	1.1
5	10–20	4	93.8	8.8	3.6	1.0
6	10–15	4	92.2	7.8	1.7	0.4
7	10–15	3	94.0	3.5	0.4	0.2
Mean			85.4		3.8	

interferences with 7-OH-MTX and APA in the previous assay methods. However, a recent comparison of several different assay methods including HPLC did not support this assumption (34). The initial phase of MTX disappearance from plasma (α-half-life) was approximately four times shorter than the β-half-life of MTX. A similar value for the α-half-life (0.75 hours) was reported for conventional doses of MTX (16).

The volume of distribution was about 18% of body weight, which approximates the extracellular space. A much greater value of 75% was reported for conventional doses of MTX (36). MTX is not tightly bound to plasma proteins. We reported a plasma protein binding of 53% ± 8% for MTX levels from 10^{-6} to 10^{-3} M (7). In previous reports MTX plasma protein binding ranged from 20% to 96% (9). In ultrafiltration experiments (7) protein binding of APA was similar to MTX (56% ± 8%), whereas 7-OH-MTX bound to plasma proteins to a greater degree (93% ± 2%).

The plasma clearance of MTX following conventional doses was reported to be in the range of 80–160 ml/minute (23–25), which favorably compares with the MTX clearance reported in this study. In contrast to the assumption of others (15), escalation of dose up to 350 mg/kg was followed by a linear increase of MTX levels in plasma.

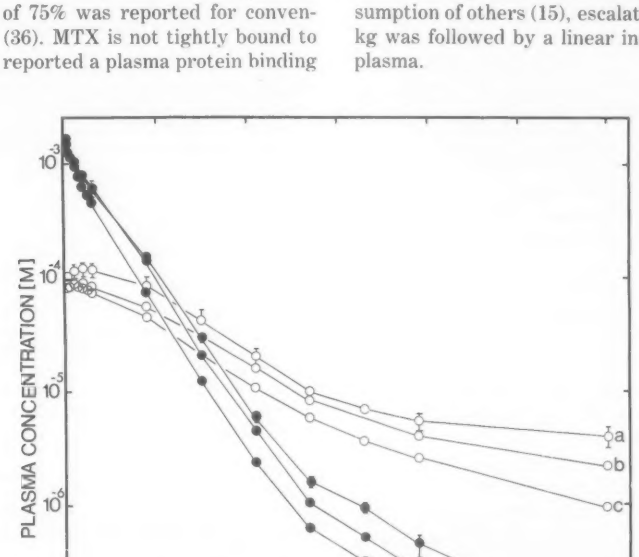


FIGURE 5.—Influence of oral application of activated charcoal on plasma kinetics of high-dose MTX. Disappearance curves of MTX (●) and 7-OH-MTX (○) are shown (patient 4). (a) Mean ± SE of 5 infusions without coadministration of activated charcoal; (b) daily application of 5 g of activated charcoal, beginning 12 hrs before MTX infusion and continuing for 3 days; and (c) daily application of 10 g of activated charcoal.

The renal clearance of MTX was approximately 84% of the total clearance of MTX, thus indicating that MTX is mainly eliminated by the kidneys, presumably by glomerular filtration and to a lesser degree by tubular secretion as suggested by others (37). The renal clearance of MTX, being about 65% of the glomerular filtration rate, was comparable to clearance values previously published for high-dose MTX (23,24) and for low-dose MTX (37,38). The constant renal clearance of MTX at plasma levels, which ranged from 10^{-4} to 10^{-7} M, supports the evidence for dose-independent elimination of MTX (up to 350 mg/kg). The cumulative urinary recovery of MTX and 7-OH-MTX in this study agrees well with the findings of others (18,39) but is inconsistent with a recovery < 60% reported in another study (24).

To our knowledge few data have been published regarding the kinetics of 7-OH-MTX (40). 7-OH-MTX peaked 3 hours after infusion of high-dose MTX (range, 12–560 μM). The plasma disappearance of 7-OH-MTX was mainly monoexponential, with a half-life about 11 times longer than the terminal half-life of the parent compound MTX. The renal clearance of 7-OH-MTX was four times smaller than the renal clearance of MTX. The urinary recovery of 7-OH-MTX indicates a significant elimination of MTX by metabolism. Similar recoveries of 7-OH-MTX were reported by other investigators (23,41), who suggested that 7-OH-MTX may contribute to the renal toxicity of high-dose MTX in man, since precipitation of the less soluble 7-OH-MTX in the renal tubules has been demonstrated to occur in monkeys (42). The large variability of 7-OH-MTX formation among patients is in contrast to the highly reproducible metabolic formation in one single patient. Repeated infusions of high-dose MTX do not provide for the assumption that hydroxylation of MTX at position 7 is an inducible metabolic step.

During and following high-dose infusions of MTX a considerable proportion of the applied dose was demonstrated to occur in the vomit, which was contaminated by bile. From our data we conclude that biliary levels of MTX and 7-OH-MTX are about 200 times higher than simultaneous plasma levels. This appears to indicate that enterohepatic circulation of MTX and its 7-hydroxy metabolite may be relevant. The excretion into the bile is a controversial issue in current literature (23,25,43,44). The enterohepatic circulation was emphasized by the influence of severe vomiting and diarrhea on plasma clearance and urinary elimination of MTX (45). Our data on the interference of coadministered activated charcoal with enterobiliary recirculation of MTX and 7-OH-MTX and its effects on plasma and renal clearances are preliminary. Further studies of biliary excretion of MTX and 7-OH-MTX in patients receiving high-dose MTX are in progress.

REFERENCES

1. Jaffe N, Frei E, III, Watts H, et al. High-dose methotrexate in osteogenic sarcoma: a 5-year experience. Cancer Treat Rep 62:259–264, 1978.
2. Rosenberg SA, Chabner BA, Young RC, et al. Treatment of osteogenic sarcoma. I. Effect of adjuvant high-dose methotrexate after amputation. Cancer Treat Rep 63:739–751, 1979.
3. Von Hoff DD, Penta JS, Helman LJ, et al. Incidence of drug-related deaths secondary to half-dose methotrexate and citrovorum factor administration. Cancer Treat Rep 61:745–748, 1977.
4. Pitman SW, Parker LM, Tattersall MHN, et al. Clinical trial of high-dose methotrexate (NSC-740) with citrovorum factor (NSC-3590)—toxicologic and therapeutic observations. Cancer Chemother Rep Part 3, vol 6:43–49, 1975.
5. Nirenberg A, Mosende C, Mehta B, et al. High-dose methotrexate with citrovorum factor rescue: predictive value of serum methotrexate concentrations and corrective measures to avert toxicity. Cancer Treat Rep 61:779-783, 1977.
6. Bleyer WA. Methotrexate: clinical pharmacology, current status and therapeutic guidelines. Cancer Treat Rev 4:87–101, 1977.
7. Breithaupt H, Kuenzlen E, and Goebel G. Rapid high pressure liquid chromatographic determination of methotrexate and its metabolite 7-hydroxymethotrexate and 2,4-diamino-N^{10}-methylpteroic acid (APA) in biological fluids. Anal Biochem 121:103–113, 1982.
8. Von Hattingberg HM, and Brockmeier D. The pharmacokinetic basis of optimal antibiotic dosage. Infection 8:21–24, 1980.
9. Paxton JW. High-dose methotrexate therapy—an area of uncertainty. Aust NZ J Med 9:722–732, 1979.
10. Salasoo S, Irving MG, Lam-Po-Tang R, et al. Methotrexate megadose followed by folate rescue. I. A study in clearance patterns. Med J Aust 1:777–779, 1976.
11. Salasoo S, Irving MG, and Freedman A. Methotrexate megadose followed by folate rescue. II. Clearance patterns in patients receiving sequential megadose infusion. Med J Aust 1:826–828, 1976.
12. Jaffe N, Frei E, III, Traggis D, et al. High-dose methotrexate with citrovorum factor in osteogenic sarcoma—progress report II. Cancer Treat Rep 61:675–679, 1977.
13. Stoller RG, Hande KR, Jacobs SA, et al. Use of plasma pharmacokinetics to predict and prevent methotrexate toxicity. N Engl J Med 297:630–634, 1977.
14. Janka GE, Wiesner H, Bidlingmaier F, et al. Hochdosierte methotrexattherapie beim osteogenen Sarkom: plasmapharmakokinetik zur vorhersage der toxizität. Klin Wochenschr 57:411–416, 1979.
15. Goldie JH, Price LA, and Harrap KR. Methotrexate toxicity: correlation with duration of administration, plasma levels, dose and excretion pattern. Eur J Cancer 8:409–414, 1972.
16. Wan SH, Huffman DH, Azarnoff DL, et al. Effect of route of administration and effusions on methotrexate pharmacokinetics. Cancer Res 34:3487–3491, 1974.
17. Frei E, Jaffe N, Tattersall MHN, et al. New approach to cancer chemotherapy with methotrexate. N Engl J Med 292:846–851, 1975.
18. Stoller RG, Jacobs SA, Drake JC, et al. Pharmacokinetics of high-dose methotrexate (NSC-740). Cancer Chemother Rep Part 3, 6(1):19–24, 1975.
19. Tattersall MHN, Parker LM, Pitman SW, et al. Clinical pharmacology of high-dose methotrexate (NSC-740). Cancer Chemother Rep Part 3, 6(1):25–29, 1975.
20. Wang Y, Lantin E, and Sutow WW. Methotrexate in blood, urine, and cerebrospinal fluid of children receiving high doses by infusion. Clin Chem 22:1053–1056, 1976.
21. Pitman SW, and Frei E, III. Weekly methotrexate-calcium leucovorin rescue: effect of alkalinization on nephrotoxicity; pharmacokinetics in the CNS; and use in CNS non-Hodgkin's lymphoma. Cancer Treat Rep 61:695–701, 1977.

22. Romolo JL, Goldberg NH, Hande KR, et al. Effect of hydration on plasma-methotrexate levels. Cancer Treat Rep 61:1393–1396, 1977.
23. Calvert AH, Bondy PK, and Harrap KR. Some observations on the human pharmacology of methotrexate. Cancer Treat Rep 61:1647–1656, 1977.
24. Isacoff WH, Morrison PF, Aroesty J, et al. Pharmacokinetics of high-dose methotrexate with citrovorum factor rescue. Cancer Treat Rep 61:1665–1674, 1977.
25. Shen DD, and Azarnoff DL. Clinical pharmacokinetics of methotrexate. Clin Pharmacokinet 3:1–13, 1978.
26. Wang Y-M. Observations on the clinical pharmacology of high-dose methotrexate in children. Cancer Bull 30:181–185, 1978.
27. Marty JJ, and Shaw J. Methotrexate pharmacokinetics: long and short infusions. Clin Exp Pharmacol Physiol 5:29–34, 1979.
28. Stoller RG, Kaplan HG, Cummings FJ, et al. A clinical and pharmacological study of high-dose methotrexate with minimal leucovorin rescue. Cancer Res 39:908–912, 1979.
29. Wang Y-M, Sutow WW, Romsdahl MM, et al. Age-related pharmacokinetics of high-dose methotrexate in patients with osteosarcoma. Cancer Treat Rep 63:405–410, 1979.
30. Evans WE, Pratt CB, Taylor RH, et al. Pharmacokinetic monitoring of high-dose methotrexate. Early recognition of high-risk patients. Cancer Chemother Pharmacol 3:161–166, 1979.
31. Oellerich M, Engelhardt P, Schaadt M, et al. Determination of methotrexate in serum by a rapid, fully mechanized enzyme immunoassay (EMIT). J Clin Chem Clin Biochem 18:169–174, 1980.
32. Donehower RC, Hande KR, Drake JK, et al. Presence of 2,4-diamino-N^{10}-methylpteroic acid after high-dose methotrexate. Clin Pharmacol Ther 26:63–72, 1979.
33. Howell SK, Wang Y-M, Hosoya R, et al. Plasma methotrexate as determined by liquid chromatography, enzyme-inhibition assay, and radioimmunoassay after high-dose infusion. Clin Chem 26:734–737, 1980.
34. Buice RG, Evans WE, Karas J, et al. Evaluation of enzyme immunoassay, radioassay, and radioimmunoassay of serum methotrexate, as compared with liquid chromatography. Clin Chem 26:1902–1904, 1980.
35. Paxton JW. Interference of 4-amino-4-deoxy-N^{10}-methylpteroic acid with radioimmunoassay of methotrexate. Clin Chem 25:491–492, 1979.
36. Henderson ES, Adamson RH, and Oliverio VT. The metabolic fate of tritiated methotrexate. II. Absorption and excretion in man. Cancer Res 25:1018–1024, 1965.
37. Huffman DH, Wan SH, Azarnoff DL, et al. Pharmacokinetics of methotrexate. Clin Pharmacol Ther 14:572–579, 1973.
38. Liegler DG, Henderson ES, Hahn MA, et al. The effect of organic acids on renal clearance of methotrexate in man. Clin Pharmacol Ther 10:849–857, 1969.
39. Pratt CB, Roberts D, Shanks EC, et al. Clinical trials and pharmacokinetics of intermittent high-dose methotrexate "leucovorin rescue" for children with malignant tumors. Cancer Res 34:3326–3331, 1974.
40. Lankelma J, Van Der Kleijn E, and Termond EFS. *In* Clinical Pharmacology of Antineoplastic Drugs, vol 1 of Applied Methods in Oncology (Pinedo HH, ed). Elsevier, 1978, pp 13–29.
41. Jacobs SA, Stoller RG, Chabner BA, et al. Dose-dependent metabolism of methotrexate in man and rhesus monkeys. Cancer Treat Rep 61:651–656, 1977.
42. ———. 7-Hydroxymethotrexate as a urinary metabolite in human subjects and rhesus monkeys receiving high-dose methotrexate. J Clin Invest 57:534–538, 1976.
43. Creaven P J, Hansen HH, and Alford DA. Methotrexate in liver and bile after intravenous dosage in man. Br J Cancer 28:589–591, 1973.
44. Strum WB, and Liem HH. Hepatic uptake, intracellular protein binding and biliary excretion of amethopterin. Biochem Pharmacol 26:1235–1240, 1977.
45. Van Den Berg HW, Murphy RF, and Kennedy DG. Rapid plasma clearance and reduced rate and extent of urinary elimination of parenterally administered methotrexate as a result of severe vomiting and diarrhea. Cancer Chemother Pharmacol 4:47–48, 1980.

Dose-Response Curves for Predicting Misonidazole-Induced Peripheral Neuropathy[1]

J. G. Schwade, R. W. Makuch, J. M. Strong, and E. Glatstein[2,*]

Using pharmacokinetics determined for 27 patients who received iv misonidazole, we have studied the relationship between various pharmacokinetic parameters and the probability of observing peripheral neuropathy. By plotting the probability of observing peripheral neuropathy as a function of each individual parameter and using a linear logistic regression model, we have found a general sigmoid response relating the observations of peripheral neuropathy to the area under the pharmacokinetic curve (AUC), the total cumulative dose throughout the course, the plasma concentration, the plasma elimination half-life, and the total cumulative AUC. By using linear logistic regression analysis, the best correlation with observing peripheral neuropathy was seen with the AUC. Statistical considerations are discussed in depth. This type of analysis may well prove useful for other toxic agents used in cancer treatment. [Cancer Treat Rep 66:1743–1750, 1982]

Recent interest in the effort to increase the susceptibility of tumors to radiation has centered on sensitizers of hypoxic cell populations. The nitroimidazoles, primarily the 5-nitroimidazole metronidazole (Flagyl) and the 2-nitroimidazole misonidazole (Ro 7-0582), have been the most promising and most studied of available compounds.

Although significant selective sensitization of hypoxic cells can be obtained both in vitro and in vivo (1,2), efficacy in patients has been demonstrated only with metronidazole and only in one study, with enhanced median survival but no cure (3). Since in vitro and in vivo studies indicate more effective sensitization with misonidazole, that drug has been selected for more intensive study of hypoxic cell radiosensitization in patients (2).

While nausea and emesis may frequently occur (3–6), peripheral neuropathy is the major dose-limiting toxic effect of misonidazole (4–7). Attempts have been made to correlate this toxicity with various pharmacologic parameters, including the total dose (4,6) and the area under the curve (AUC) in which misonidazole plasma levels are plotted as a function of time (4).

We have determined certain misonidazole pharmacologic parameters in patients after iv administration of the drug and have applied a unique statistical analysis to determine whether any correlation exists between these measurable parameters and the observed neurotoxicity. Our findings indicate that AUC shows the best correlation and should be of value in identifying those individuals at greatest risk for peripheral neuropathy after misonidazole administration.

MATERIALS AND METHODS

Plasma pharmacokinetics were determined in 27 patients who received misonidazole. Formulated drug was provided by the Division of Cancer Treatment, National Cancer Institute (Bethesda, MD), lyophilized in 500-mg amber vials, and reconstituted with 19.5 ml of normal saline. Infusion was carefully timed over 5 minutes. The drug was infused into a vein in an upper extremity, and at least three plasma samples were obtained in 1–24 hours following the start of infusion. Misonidazole was administered in doses of 1.5 or 2.0 g/m^2 twice weekly for five to 14 doses (tables 1 and 2). Toxicity consisted of sensory peripheral neuropathy (13 patients), nausea and/or emesis (eight), and tinnitus (two). Toxicity was mild and self-limited in all but two patients, who were hospitalized because of severe peripheral neuropathy (table 2).

Plasma levels of misonidazole and desmethylmisonidazole were determined by high-pressure liquid chromatography, using a modification of the method described by Workman et al (8). Patients received misonidazole either as part of a phase I trial or as part of an ongoing randomized clinical trial testing the efficacy of the drug with irradiation in patients with carcinoma of the esophagus or locally unresectable osteosarcoma. All patients signed informed consent forms.

[1]Received Dec 18, 1981; revised May 28, 1982; accepted June 30, 1982.

[2]Radiation Oncology Branch (*J. G. Schwade* and *E. Glatstein*), Biometric Research Branch (*R. W. Makuch*), and the Laboratory of Chemical Pharmacology (*J. M. Strong*), Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

**Reprint requests to:* Eli Glatstein, MD, Radiation Oncology Branch, Division of Cancer Treatment, National Cancer Institute, Bldg 10, Rm B3B38, Bethesda, MD 20205.

TABLE 1.—Occurrence of peripheral neuropathy in patients who received misonidazole iv twice/wk*

Patient No.	n	C_0 (μg/ml)	CV (%)	$T_{1/2}$ (hrs)	CV (%)	AUC (μg·hr/ml)	CV (%)	Dose/ fraction (g)	Total dose (g)	No. of doses	Total AUC (μg·hr/ml)
						1.5 mg/m²					
1	1	63.3		10.9		1000		2.55	25.5	10	10,000
2	1	82.5		8.9		1060		2.3	11.5	5	5300
3	6	72.8	14	8.7	6	912	13	3.0	30.0	10	9120
4	1	62.3		15.3		1375		2.7	27.0	10	13,750
5	1	58.2		8.1		677		2.25	11.25	5	3385
6	1	58.7		7.4		735		2.7	27.0	10	7350
7	2	63.1	11	8.6	7	781	5	2.5	25.0	10	7810
8	1	68.8		7.4		735		2.6	26.0	10	7350
9	5	56.0	12	9.3	1	753	13	2.7	27.0	10	7530
10	1	86.2		8.9		1107		2.3	11.5	5	5535
11	4	74.9	3	5.2		566	10	2.5	25.0	10	5660
12	1	60.6		8.8		770		3.0	15.0	5	3850
Median		63.2		8.8		776		2.58	25.5	10	7350
Range		56.0–86.2		5.2–15.1		566–1375		2.25–3.0	11.3–30.0	5–10	3385–13,570
						2.0 mg/m²					
13	1	72.4		10.7		1118		3.5	17.5	5	5590
14	1	59.0		8.2		698		2.5	12.5	5	3490
15	4	90.1	28	9.0	9	1150	19	3.25	22.8	7	8050
Median		72.9		9.0		1118		3.25	17.5	5	5590

*CV = coefficient of variation, n = No. of determinations.

TABLE 2.—Absence of peripheral neuropathy in patients who received misonidazole iv twice/wk*

Patient No.	n	C_0 (μg/ml)	CV (%)	$T_{1/2}$ (hrs)	CV (%)	AUC (μg·hr/ml)	CV (%)	Dose fraction (g)	Total dose (g)	No. of doses	Total AUC (μg·hr/ml)
						1.5 mg/m²					
16	1	58.8		8.8		745		2.6	15.6	6	4470
17	1	87.2		8.4		1060		3.6	27.6	7	7420
18	1	61.3		16.3		1442		2.8	22.4	8	11,536
19	8	63.4	8	9.5	50	917	43	3.3	42.9	13	11,921
20	1	75.7		14.6		1591		3.46	24.2	7	11,137
21	8	70.8	12	10.1	8	1024	6	2.8	28.0	10	10,240
22	4	68.2	5	10.3	14	1012	9	3.0	30.0	10	10,120
23	2	80.0	15	11.0	7	1259	8	2.6	20.8	8	10,072
Median		69.5		10.2		1042		2.9	25.9	8	10,180
Range		58.8–87.2		8.4–16.3		745–1591		2.6–3.6	15.6–42.9	6–13	4470–11,921
						2.0 mg/m²					
24†								2.4	16.8	7	
25	3	123	16	9.5	17	1718	28	3.25	22.8	7	12,026
26†								2.9	26.1	9	
27	4	104	17	14.1	6	2121	13	3.6	25.2	7	14,847
28	1	92		12.3		1644		3.5	35.0	10	16,440
29	1	79.6		10.3		1180		4.0	32.0	8	9440
Median		98		11.3		1681		3.39	25.7	7.5	13,437
Range		77.6–123		9.5–14.1		1180–2121		2.4–4.0	16.8–35.0	7–10	9440–16,440

*CV = coefficient of variation, n = No. of determinations.
†Patient excluded from all calculations.

Statistical Methods

For each parameter examined, the median and range were determined within the patient groupings defined by the dose of misonidazole (1.5 g/m^2 or 2.0 g/m^2) and by whether a peripheral neuropathy was present. A blocked Wilcoxon rank-sum test adjusted for ties, with the dose of misonidazole as the blocking factor, was used to compare the value of each parameter between patients who developed or did not develop peripheral neuropathy. The normal approximation was used to calculate two-sided significance probabilities (9).

A linear logistic regression model and maximum likelihood techniques (10) were used to characterize the relationship between each parameter and the probability of having peripheral neuropathy. The actual dose of misonidazole was included as a covariate in each regression analysis. Statistical significance of the covariate was evaluated in each analysis, using standard normal theory to determine whether it was significantly different from 0. If it was not, then a simpler expression resulted, with only one independent variable. One consequence is that the relation between each parameter and the probability of developing peripheral neuropathy can be illustrated graphically. A goodness-of-fit test for each logistic regression model (11) was computed to ensure that the data were described adequately by the model. Using the patient's observed parameter value, the fitted regression model was then employed to classify a patient into one population (peripheral neuropathy) or another (no peripheral neuropathy). Using the fitted regression model, perfect patient discrimination occurs when each patient is correctly predicted to have or not to have peripheral neuropathy. Such perfect prediction is unexpected for a number of reasons, including patient heterogeneity. To quantify the extent of departure from perfect discrimination, a misclassification error probability was calculated. It is defined as the ratio of the number of patients who were classified incorrectly using the regression model and the total number of patients evaluated. Since this naive projection tends to underestimate the true misclassification error rate, the "leave-one-out jackknife" (12) was used to provide less-biased error probabilities.

Pharmacokinetic Analysis

The pharmacokinetic parameters used in this analysis were determined after obtaining at least three blood samples drawn during the 1–24-hour interval after iv administration of misonidazole. A least squares analysis was used to fit the data to a single compartment.

Pharmacokinetic Model

The curve plotting misonidazole plasma levels as a function of time was best described by a biexponential function following a 5-minute iv injection. However, as previously reported (5), the initial distribution phase had a mean half-life of 5.8 minutes. Therefore, for the purposes of this study, only the elimination phase was evaluated, since the pharmacokinetic parameters considered in this report would not be significantly affected by the early distribution phase. All parameters were calculated from the misonidazole plasma levels using the relationship $C_t = C_0e^{-\beta t}$ (equation 1), where C_t(μg/ml) is the misonidazole plasma level at time t (minutes), C_0 (μg/ml) is the apparent plasma misonidazole concentration at time equal to 0, and β (hour^{-1}) is the slope of the best-fit line to data based on equation 1 (fig 1). The misonidazole plasma elimination half-life $T_{1/2}$ (hour) was determined from the relationship: $T_{1/2} = \ln 2/\beta$ (equation 2). An estimate of the area under the plasma misonidazole concentration-time curve (AUC) was made using the following equation: $AUC = C_0/\beta$ (equation 3).

When the AUC is determined using equation 3, plasma misonidazole levels obtained before 1 hour after drug administration should not be included in the calculation. This is to ensure that the drug has reached equilibrium between the plasma and its major body tissue compartments.

Alternate methods for determining AUC, such as the trapezoidal rule (13), may be applied, especially when the drug is administered orally. However, a much larger number of misonidazole plasma level determinations

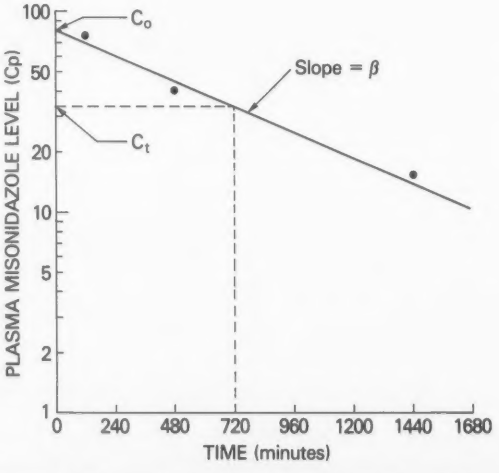


FIGURE 1.—Pharmacokinetic plot of plasma misonidazole levels as function of time (*see* text).

would be required to obtain a good estimate of the AUC, following administration by the oral route.

While multiple AUC determinations were obtained for one patient, a "total AUC" was determined in other patients by multiplying the mean AUC by the number of administered doses. Thus, for most patients, total AUC represents an approximation. Detailed pharmacokinetics, including levels in plasma, pleural fluid, and tumor, have previously been reported in selected patients (5).

RESULTS

Values of four pharmacologic parameters, C_0, $T_{1/2}$, AUC, and total AUC, were compared between patients who developed or did not develop peripheral neuropathy. Those with peripheral neuropathy tended to have higher C_0 than patients without peripheral neuropathy ($P = 0.08$). The values for the remaining three parameters were significantly higher in patients with peripheral neuropathy ($P = 0.013$, 0.005, and 0.004, respectively). Similar analyses were performed to compare the two groups for total dose and the number of given doses. Total dose tended to be higher in patients with peripheral neuropathy compared with those without peripheral neuropathy ($P = 0.066$). The number of doses given was not significantly associated with the development of peripheral neuropathy ($P = 0.27$).

The association between each of the above parameters and the probability of peripheral neuropathy was determined using the linear logistic model with misonidazole in g/m^2 as a covariate. To carry out the analysis, patients were divided into one of two groups. For patients who developed peripheral neuropathy, a value of 1 was assigned as the dependent variable; patients who did not develop peripheral neuropathy were assigned a value of 0. A clear dose-response relationship was found between AUC and the probability of developing peripheral neuropathy (fig 2). The covariate, dose, was excluded from the equation since it was not significantly different from 0 ($P = 0.47$). The parameter AUC was significantly different from 0 ($P = 0.016$). Similar findings were also evident for $T_{1/2}$ (fig 3), total AUC (fig 4), and C_0 (fig 5). However, the covariate was of some importance ($P = 0.15$) in the regression equation involving total dose, and the two parameters were highly correlated with one another. Thus, the parameter total dose was eliminated from further consideration since a single graphic display and any clear interpretation of the parameter were complicated by the necessary inclusion of the covariate in the regression model.

The fitted regression model for each parameter is indicated below each figure. The equation can be used to predict whether a patient with a specified parameter value, X_* (eg, the patient's AUC), will develop peripheral neuropathy. Specifically, the parameter value, X_*, is inserted in the equation $\hat{\alpha} + \hat{\beta}X$, and if the resultant quantity is ≤ 0, then the probability of the occurrence of peripheral neuropathy predicted from the model is ≤ 0.50. Since patients without peripheral neuropathy were assigned initially a value of 0 for the dependent variable in the regression model, a reasonable classification rule is that the model predicts that a patient with parameter value of X_* will not develop peripheral neuropathy if $\hat{\alpha} + \hat{\beta}X_* \leq 0$. Conversely, if the quantity $\hat{\alpha} + \hat{\beta}X_* > 0$, then the model predicts that peripheral neuropathy will develop in the patient.

To illustrate, suppose the parameter AUC is used to predict whether patients will or will not develop peripheral neuropathy. The equation is $y = \hat{\alpha} + \hat{\beta}X =$

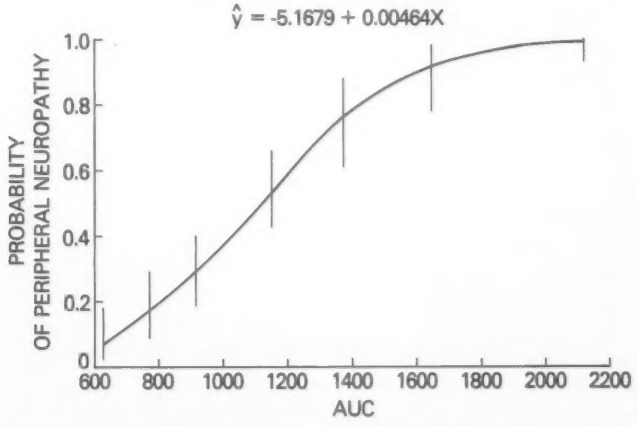


FIGURE 2.—Dose-response curve, using linear logistic model; shows probability of developing peripheral neuropathy as function of area under pharmacokinetic curve. Error bars represent ± 1 SE.

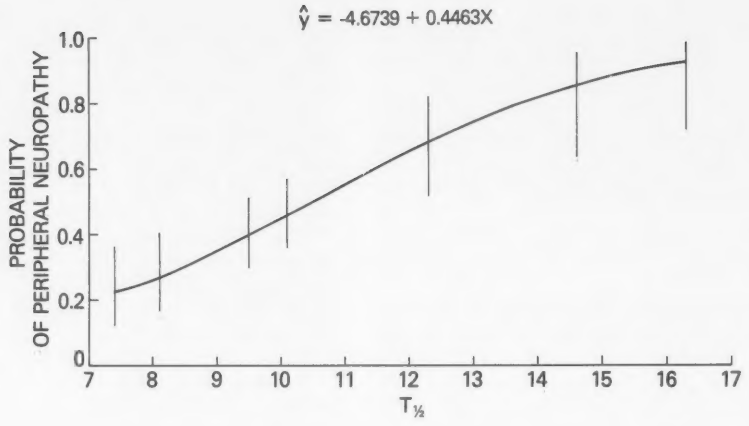


FIGURE 3.—Dose-response curve using linear logistic model for probability of developing peripheral neuropathy as function of half-life. Error bars represent ± 1 SE.

$-5.1679 + 0.00464X$ (fig 2). Then $y \leq 0$ whenever $X \leq 1113$. This implies that the model is used to predict that a patient will not have peripheral neuropathy if the AUC is ≤ 1113. The data in table 1 indicate that the model's prediction is incorrect for patients 4, 13, and 15, since their values exceed 1113 and yet it is known that they did not have peripheral neuropathy. Likewise, the model predicts that a patient will have peripheral neuropathy if the AUC exceeds 1113. Table 2 shows that the model incorrectly predicted the status of patients 16, 17, 19, 21, and 22.

The sum of these two types of misclassification divided by the total number of patients provides a misclassification error rate estimate of (3 + 5)/27 = 29.6% if AUC is used. Error rate estimates are calculated in a similar manner for the other parameters; they are 37.0%, 14.8%, and 33.3% for $T_{1/2}$, total AUC, and C_0, respectively. These estimates of the true misclassification error rate may be too low since the same data were used to obtain the fitted regression model. For this reason the jackknife method, a cross-validation technique, was used to obtain less-biased error rate

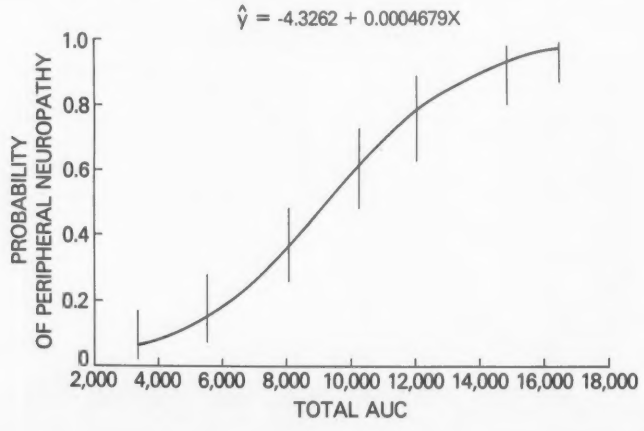


FIGURE 4.—Dose-response curve, using linear logistic model for probability of developing peripheral neuropathy as function of total area under pharmacokinetic curve (AUC × total No. of injections). Error bars represent ± 1 SE.

estimates. This method gives error rate estimates of 33.3%, 37.0%, 22.2%, and 33.3% for AUC, $T_{1/2}$, total AUC, and C_0, respectively.

Goodness-of-fit tests indicated that the final regression model for each parameter summarized the observed data quite well since the two-sided P values exceeded 0.40 in each instance. Although fit is one criterion for evaluating a risk function, it is also desirable for the risk slope to be steep. A steep slope indicates that the patients are classified correctly by the model with a high degree of success. Visual inspection of figures 2–5 suggests that AUC and total AUC are the best candidates for discriminating effectively in regard to the frequency of peripheral neuropathy.

DISCUSSION

The clinical utility of hypoxic cell radiosensitizers is still unproved. To evaluate these compounds effectively, maximally tolerated plasma levels should be obtained to achieve optimal concentrations of sensitizers in the region of hypoxic cells at the time of irradiation. Additionally, in patients with resistant strains of *Trichomonas vaginalis*, which is relatively resistant to metronidazole, use of adequate doses of misonidazole may require knowledge of individual susceptibility to toxicity (14,15). While some authors support a strong correlation between neurotoxicity and total dose (4,6), our results and those of Walker and Strike (7) do not support this finding. At best, total dose is only a crude indicator of pharmacokinetic parameters, mainly AUC, $T_{1/2}$, and peak plasma level (C_0 in this case). While total dose may be useful in obtaining information to determine starting doses for a course of therapy, it has little value in predicting toxic effects for individual patients. Determination of pharmacokinetic parameters such as AUC, $T_{1/2}$, and C_0 does allow identification of an individual's probability of developing peripheral neuropathy. AUC, $T_{1/2}$, and C_0 can easily be determined from three or more plasma levels obtained 1–24 hours after administration of the initial dose and at intervals during the course of therapy. A total AUC can be estimated by multiplying AUC by the number of planned doses, which gives an estimate of the probability of peripheral neuropathy with the planned dose. Appropriate adjustments in dose can thus be made or courses of therapy can be lengthened or abbreviated, in accordance with dose-response curves. While this paper contains dose-response data only for misonidazole toxicity, similar information might be useful for other drugs used in cancer therapy, particularly cytotoxic agents that are thought to have dose-response relationships for tumor response and that undoubtedly have individual variation in therapeutic ratio.

REFERENCES

1. CHAPMAN JD. Hypoxic sensitizers—implications for radiation therapy. N Engl J Med 301:1429–1432, 1979.
2. FOWLER JF, and DENEKAMP J. A review of hypoxic cell radiosensitization in experimental tumors. Pharmacol Ther 7:413–444, 1979.
3. URTASUN R, BAND P, CHAPMAN JD, ET AL. Radiation and high dose metronidazole in supratentorial glioblastomas. N Engl J Med 294:1364–1367, 1976.
4. DISCHE S, SAUNDERS MI, LEE ME, ET AL. Clinical testing of the radiosensitizer, Ro-07-0582: experience with multiple doses. Br J Cancer 35:567–579, 1977.
5. SCHWADE JG, GANGJI D, and STRONG JM. IV misonidazole (NSC-261037)—report of initial clinical experience. Cancer Clin Trials 4:33–39, 1981.

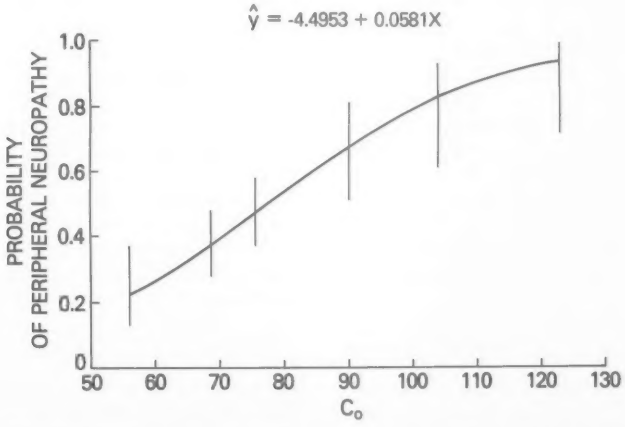


FIGURE 5.—Dose-response curve, using linear logistic model for probability of developing peripheral neuropathy as function of apparent plasma concentration at time = 0 (*see* text). Error bars represent ± 1 SE.

6. WASSERMAN TH, PHILLIPS TL, JOHNSON RJ, ET AL. Initial United States pharmacologic evaluation of misonidazole (Ro-07-0582), a hypoxic cell radiosensitizer. Int J Radiat Oncol Biol Phys 5:775–786, 1979.
7. WALKER MD, and STRIKE TA. Misonidazole peripheral neuropathy—its relationship to plasma concentration and other drugs. Cancer Clin Trials 3:105–109, 1980.
8. WORKMAN P, LITTLE CJ, MARTEN PR, ET AL. Estimation of the hypoxic cell sensitizer misonidazole and its *O*-demethylated metabolite in biological materials by reversed-phase and high-performance liquid chromatography. J Chromatogr 145:507–512, 1975.
9. LEHMANN EL. Nonparametrics: Statistical Methods Based on Ranks. San Francisco, CA, Holden-Day, Inc, 1975.
10. COX DR. Analysis of Binary Data. London, England, Methuen and Co, Ltd, 1970.
11. TSIATIS AA. A note on a goodness-of-fit test for the logistic regression modes. Biometrika 67:250–251, 1980.
12. MILLER RG. The jackknife—a review. Biometrika 61:1–15, 1979.
13. GIBALDI M, and PERRIER D. Drugs and the pharmaceutical sciences. *In* Pharmacokinetics (Swarbrick J, ed). New York, NY, Marcel Dekker, 1975, vol 1, appendix 4, pp 293–296.
14. GOLDMAN P. Reply to letter to the editor. N Engl J Med 304:735, 1981.
15. MEINGASSNER J, and THURNER J. Strain of *Trichomonas vaginalis* resistant to metronidazole and other 5-nitroimidazoles. Antimicrob Agents Chemother 15:254, 1979.

Metabolism and Mechanism of the Antileukemic Action of Isomeric Aryldimethyltriazenes[1,2]

G. Sava,* T. Giraldi, L. Lassiani, and C. Nisi[3,4]

Mice bearing TLX5 lymphoma or P388 leukemia were treated with *ortho*, *meta*, and *para* isomers of the salts of (3,3-dimethyl-1-triazeno)benzoic acid. Survival time was markedly increased in mice given the *para* isomer; effects were less pronounced for the *meta* isomer and absent for the *ortho* isomer. The in vivo effects of the tested compounds did not correlate either with the propensity of the drugs to undergo oxidative *N*-demethylation and hydrolysis to diazonium cations or with in vitro cytotoxicity for TLX5 lymphoma cells. The *para* isomer did not reduce the number and viability of peritoneal TLX5 lymphoma cells after in vivo and in vitro treatment, whereas a dose-dependent reduction that can even result in the absence of clonogenic tumor cells occurred in the brains of the treated animals. These data indicate that the increased survival time of the tumor-bearing mice treated with the *para* isomer should not be ascribed to cytotoxic effects of the drug and might be attributed to inhibition of tumor cell dissemination in various organs of the host, as already observed for solid metastasizing tumors in mice. [Cancer Treat Rep 66:1751–1755, 1982]

The imidazole dimethyltriazene dacarbazine displays antitumor activity against several rodent transplantable tumors (1) and is also usefully employed in clinical practice as a single drug or integrated into combined modalities for the treatment of malignant melanoma and other tumors in man (2–4). The structurally related aryldimethyltriazenes also display antitumor activity in experimental systems, in some instances equal to or greater than that displayed by dacarbazine (5–7). Evidence has been provided in experimental systems that dimethyltriazenes require metabolic activation to exert their antitumor cytotoxic activity (5). Monomethyltriazenes produced via the formation of 3-hydroxy-3-methyltriazene by microsomal oxidative *N*-demethylation of the parent dimethyl compounds have been suggested as being the active metabolites (8–10). Yet, examination of the measurements performed on in vitro demethylation and in vivo antitumor activity for several dimethyltriazenes (11,12) shows no apparent correlation between them. A possible active role of the diazonium cations derived by hydrolysis has been ruled out, since dimethyltriazenes stable in aqueous solution for the length of the experiments have good antitumor activity (5) and since no antitumor effects result from the in vivo administration of aryldiazonium cations (5,13).

Aryldimethyltriazenes having different substituents on the benzene ring have mainly been used so far in investigating the structure-activity relationships of this group of drugs. In the present investigation the *ortho* (*o*-DM-COOK), *meta* (*m*-DM-COONa), and *para* (*p*-DM-COOK) isomers of the salts of (3,3-dimethyl-1-triazeno)benzoic acid, having different steric properties but closely similar lipophilicity, have been examined. *o*-DM-COOK and *m*-DM-COONa are isomeric derivatives of *p*-DM-COOK, which has already been found to prolong the survival time of leukemic mice (14) and to markedly suppress the metastatic spread of solid mouse tumors (13,15). The in vitro cytotoxicity for TLX5 lymphoma cells has thus been compared with the in vivo increase of survival time of mice implanted ip with the same tumor or with P388 leukemia. Furthermore, the half-life for hydrolysis to diazonium cations in aqueous solution at neutral pH and the oxidative *N*-demethylation by 10,000 x *g* rat liver supernatant for the three compounds used have been determined in order to examine possible relationships between these parameters and antitumor effects. Finally, the effects of the treatment with *p*-DM-COOK on the growth and viability of peritoneal tumor cells, as well as on the presence of clonogenic tumor cells in the brains of TLX5 lymphoma-bearing mice, have been determined.

[1]Received Dec 15, 1981; revised May 18, 1982; accepted June 16, 1982.

[2]Supported by Italian National Research Council—Special Project "Control of Neoplastic Growth" (contract 80.01562.96) and by a special grant from the University of Trieste.

[3]Istituto di Farmacologia (*G. Sava* and *T. Giraldi*) and Istituto di Chimica Farmaceutica (*L. Lassiani* and *C. Nisi*), Università di Trieste, Italy.

[4]We acknowledge the skillful technical assistance of Dr. Rinalda Campanini.

*Reprint requests to: Dr. Gianni Sava, Università degli Studi di Trieste, Istituto di Farmacologia, Via A. Valerio, N. 32, I-34100 Trieste, Italy.

MATERIALS AND METHODS

The synthesis of the compounds used was performed following already reported procedures (16).

In Vitro Cytotoxicity

For the determination of the in vitro cytotoxic effects of the triazenes in the presence of metabolic activation, a postmitochondrial liver supernatant, obtained by centrifuging a liver homogenate at 10,000 × *g* for 30 mins, was used. The livers were obtained from Sprague-Dawley rats, which received 0.5 g/liter of phenobarbital in their drinking water for 4 days before being killed. The livers were homogenized in 4 volumes of 0.1 M potassium phosphate buffer (pH 7.4), using a Potter-Elvehjem tissue grinder. Protein concentration, determined by the method of Lowry et al (17) with bovine serum albumin as the standard, was adjusted to 40 mg/ml. The incubation mixture consisted of 1 ml of the buffer containing the drug, 20 mg of protein from the liver postmitochondrial supernatant, 12 μmols of $MgCl_2$, 0.8 μmol of NADP, 16 μmols of glucose-6-phosphate, and 0.35 unit of glucose-6-phosphate dehydrogenase (grade II from Boehringer-Mannheim), which was added to 3 ml of Dulbecco culture medium containing 4 × 10^6 TLX5 lymphoma cells and supplemented with 4% (vol/vol) fetal calf serum. After 2 hrs of incubation at 37°C with gentle shaking, cell viability was bioassayed by injecting 0.1-ml aliquots of the incubation mixture into groups of five animals whose survival time was recorded. These experiments were also performed without metabolic activation, omitting the liver supernatant and cofactors.

In Vitro Oxidative *N*-Demethylation and Hydrolysis

The incubation mixture was the same as above with the exception that the 3 ml of Dulbecco culture medium containing the tumor cells was replaced with 0.1 M potassium phosphate buffer (pH 7.4) containing 100 μmols of semicarbazide. After incubation for 2 hrs at 37°C with gentle shaking, the reaction was stopped by the addition of 1 ml of 20% (wt/vol) $ZnSO_4$ and 1 ml of saturated $Ba(OH)_2$ solution. After centrifugation, the Nash colorimetric assay for formaldehyde was carried out on the supernatant (18); the demethylation capacity of the system was checked by using aminopyrine as the substrate and was linear for at least 2 hrs.

The kinetic law of decomposition of the triazenes used, studied at pH 7.0 and 37°C in 65 mM phosphate buffer, was pseudo first-order with respect to the triazene concentration in all cases; the procedure was that reported by Kolar and Preussmann (19).

In Vivo Experiments

Approximately 10^5 TLX5 lymphoma cells or 10^6 P388 leukemia cells were transplanted ip into groups of five to ten CBA/lac or BDF_1 hybrids, respectively. The treatment was performed ip as indicated, using drugs freshly dissolved in 0.1 N $NaHCO_3$ and administered in 0.1 ml/10 g of animal weight. The survival time of the animals was recorded, and the total number of peritoneal tumor cells was measured at the end of treatment of TLX5 lymphoma-bearing mice. This was done by means of a Coulter Counter model ZF, after careful collection of tumor cells by repeated washing of the peritoneal cavity with isotonic NaCl solution.

The viability of peritoneal tumor cells at the end of treatment with repeated administrations of *p*-DM-COOK in mice bearing TLX5 lymphoma was bioassayed by ip transplantation of 10^5 tumor cells from the treated donors into normal recipients. The presence of clonogenic tumor cells in the brain was also measured at the end of treatment by transplanting the whole brains sc into normal mice. In both cases the survival time of the normal untreated recipients was recorded, which provided an indirect indication of the viability or number of tumor cells present in the bioassayed sample (20).

RESULTS

In Vivo Antitumor Activity

The effects of the three isomers on the survival time of CBA/lac mice bearing TLX5 lymphoma or of BDF_1 hybrids bearing P388 leukemia are reported in table 1. Each compound was tested using either single or repeated doses, including maximum tolerated doses. Both tumors were unresponsive to the treatment with *o*-DM-COOK with all the doses and treatment schedules employed. The antitumor activity of *p*-DM-COOK, in terms of the number of dose levels causing significant increases of survival time of the treated animals, was pronounced for both tumors; the activity of *m*-DM-COONa, though evident, was less marked.

The number of peritoneal tumor cells present when the animals were killed was not significantly modified by *p*-DM-COOK. The viability of the same tumor cells, bioassayed by transplantation into normal mice, was slightly reduced at the two higher doses employed. On the other hand, the survival time of mice transplanted with whole brains of the treated animals was significantly increased with all the doses used. The percent of animals having no detectable clonogenic tumor cells in their brains increased from 0% to 100% with increasing doses (table 2).

In Vitro Cytotoxicity

The cytotoxic activity of the tested compounds for TLX5 lymphoma cells, after 2 hrs of incubation in vitro

TABLE 1.—Effects of tested compounds on survival time of mice bearing TLX5 lymphoma or P388 leukemia

Tumor line	Compound	Treatment schedule	Active doses (mg/kg/day)*	Survival time (% T/C)†
TLX5 lymphoma	*o*-DM-COOK	Daily on Days 3–7	Inactive	
		Day 1 only	Inactive	
	m-DM-COONa	Daily on Days 3–7	100	146
		Day 1 only	200	128
	p-DM-COOK	Daily on Days 3–7	200,100,50,25	171,170,145,126
		Day 1 only	300	133
P388 leukemia	*o*-DM-COOK	Daily on Days 1–5	Inactive	
		Day 1 only	Inactive	
	m-DM-COONa	Daily on Days 1–5	200	187
		Day 1 only	200	134
	p-DM-COOK	Daily on Days 1–5	200,100,50	157,140,129
		Day 1 only	400,200	166,143

*Toxic deaths do not occur at the indicated active doses, whereas they are observed at the next unreported higher dose. For *o*-DM-COOK, toxic doses are 25–50 mg/kg.

† % T/C is the % ratio of the average survival time for each treated group to that of relevant controls. Only values significantly different from controls by the Student-Newmann-Keuls test (25) ($P < 0.05$) are reported.

TABLE 2.—Effects of *p*-DM-COOK on survival time and No. and viability of tumor cells in mice bearing ip TLX5 lymphoma*

Daily dose (mg/kg)	Survival time (days), mean ± SE	No. ($\times 10^6$) of peritoneal tumor cells when killed, mean ± SE	Survival time (days) of mice inoculated with—		Survival at 4 mos†
			10^5 peritoneal tumor cells	Whole brains	
0	10.2 ± 0.2	697 ± 68	10.1 ± 0.3	10.8 ± 0.3	0/14
12.5	10.3 ± 0.3	501 ± 92	10.8 ± 0.2	14.0 ± 0.6‡	0/5
25	16.7 ± 1.2‡	457 ± 86	11.6 ± 0.4	20.7 ± 4.7‡	2/5
50	18.0 ± 1.5‡	471 ± 104	12.1 ± 0.2	15.3 ± 0.5‡	5/12
100	14.7 ± 0.3‡	546 ± 64	13.0 ± 0.2‡	21.6 ± 2.1‡	8/13
200	12.3 ± 0.3	574 ± 77	14.2 ± 0.6‡	120	5/5

*Groups of 5–14 tumor-bearing mice were treated ip with *p*-DM-COOK on Days 3–7 after tumor transplantation; 24 hrs after the last drug administration the animals were killed and their peritoneal tumor cells and whole brains were transplanted into normal recipients whose survival time is reported.

†No. of mice surviving at 4 mos/total No. transplanted with whole brains.

‡Significantly different from values for controls by the Student-Newmann-Keuls test (25) ($P < 0.05$).

at 37°C, is reported in figure 1. All three isomers were devoid of any cytotoxic effect for tumor cells when the incubation was performed in the presence of 10,000 × *g* rat liver supernatant and cofactors. When the drugs were added to the tumor cell suspension in the absence of metabolic activation, only *o*-DM-COOK at the two higher concentrations caused a significant cytotoxicity as evidenced by prolonged survival of the tumor recipients.

In Vitro Oxidative *N*-Demethylation and Hydrolysis

The percent of demethylation of the tested compounds, expressed as mols of formaldehyde produced during the in vitro incubation over the molar starting amount of dimethyltriazene, is reported in table 3. A 10%–13% demethylation was observed for *o*-DM-COOK, depending on the concentration of the drug used. *p*-DM-COOK was only weakly demethylated, whereas *m*-DM-COONa was almost completely resistant to in vitro oxidative *N*-demethylation.

The kinetic constants of hydrolysis to diazonium cations for the tested compounds are also reported in table 3. *o*-DM-COOK rapidly hydrolyzed in aqueous solution at neutral pH and 37°C; 64.8% of the initial amount was converted to diazonium cations within 2 hrs. *m*-DM-COONa and *p*-DM-COOK were markedly more resistant to hydrolysis; their relevant values were 7.7% and 2.8%, respectively.

DISCUSSION

The antitumor activity of the isomers of the salts of (3,3-dimethyl-1-triazeno)benzoic acid observed after in vivo administration to mice bearing either of two tumor

TABLE 3.—In vitro demethylation and kinetic parameters of hydrolysis of the tested compounds

Compound	Starting concentration, μg/ml	% demethylation, mean ± SE*	K × 10^{-3} (min^{-1})	Half-life, mins
o-DM-COOK	400	12.6 ± 4.8	8.710	79.58
	800	9.8 ± 2.3		
m-DM-COONa	400	1.5 ± 0.6	0.668	1037.74
	800	0.9 ± 0.5		
p-DM-COOK	400	3.0 ± 1.3	0.240	2889.46
	800	2.0 ± 0.8		

*Values were obtained from 4 separate determinations.

lines ranged from the inactivity of *o*-DM-COOK to the pronounced activity of *p*-DM-COOK; the activity of *m*-DM-COONa fell in between. For *p*-DM-COOK, the number of peritoneal tumor cells found at the end of the treatment in mice bearing TLX5 lymphoma was not significantly reduced, and their viability was slightly decreased only at the highest and nonoptimal doses. Considering the growth kinetics of this tumor, these reductions cannot be held responsible for the increase in survival time caused by *p*-DM-COOK. Indeed, a purely cytotoxic agent, cyclophosphamide, given at doses that increased the survival time of the mice to an extent similar to that caused by *p*-DM-COOK, caused the absence of detectable peritoneal tumor cells (14).

The lack of significant cytotoxicity of *p*-DM-COOK was confirmed when TLX5 tumor cells were incubated in vitro with the drug, either in the presence or absence of metabolic activation. The absence of significant cytotoxic effects has also been observed in cell cultures of Lewis lung carcinoma treated with 1.2 mg/ml of *p*-DM-COOK for several hours (unpublished results). *m*-DM-COONa was similarly devoid of cytotoxicity in vitro for TLX5 tumor cells , whereas *o*-DM-COOK significantly reduced the viability of TLX5 tumor cells after incubation in vitro in the absence of metabolic activation. This finding, however, should be interpreted in view of the fact that this compound has a half-life for hydrolysis to diazonium cations that is much shorter than that of its analogs examined here. Appreciable amounts of cytotoxic diazonium cations (11) can thus be produced during incubation only from this substance. The measurements of the oxidative *N*-demethylation of the tested isomers should be regarded as indicative only, since a complex kinetics of demethylation, presumably due to inhibition of the enzymatic system by the drugs, has been observed for dimethyltriazenes (12,21,22).

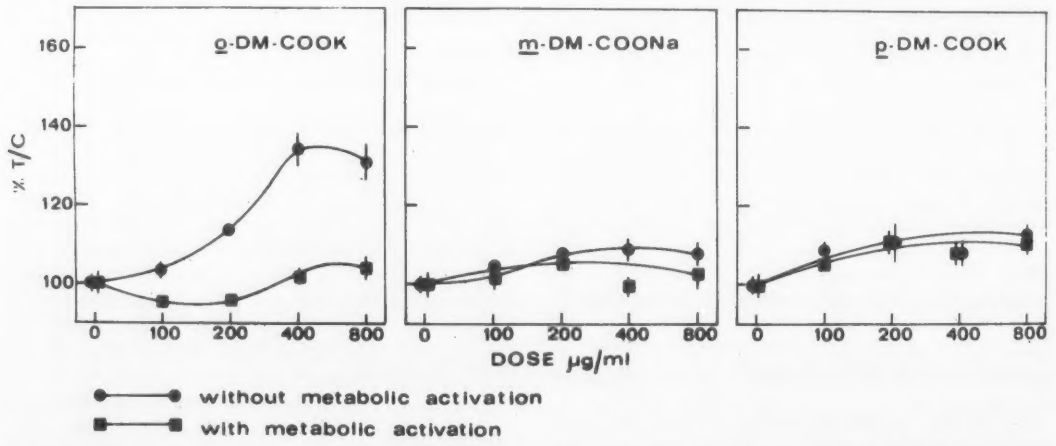


FIGURE 1.—In vitro cytotoxicity of the 3 isomeric derivatives of (3,3-dimethyl-1-triazeno)benzoic acid. Cytotoxic effects of the tested dimethyltriazenes result in increased survival time of the normal recipients inoculated with tumor cells treated in vitro, as compared with survival time of control animals receiving untreated tumor cells. Results obtained in 4 separate experiments have been averaged and are expressed as the % ratio (% T/C) of the survival time of mice receiving treated tumor cells over that for controls. Statistical analysis by Student-Newmann-Keuls test (25).

However, *o*-DM-COOK, though it is more subject to *N*-demethylation than are its isomers, was not cytotoxic in vitro in the presence of metabolic activation. These data indicate a lack of correlation between an increase in survival time of mice bearing tumors treated in vivo and the propensity of the isomers examined to generate diazonium cations or to undergo *N*-demethylation. It would thus appear that the in vivo effects of DM-COOK isomers are independent of the metabolic activation through the two pathways previously indicated and that these drugs might act directly on tumor cells.

As far as the mechanism involved in increasing the survival time of mice treated in vivo, a remarkable reduction of clonogenic tumor cells was observed in the brains of the treated animals. The ip administration of the drug did not significantly reduce the viability and number of peritoneal tumor cells, but the drug might prevent metastatic tumor cells from reaching the brain. Indeed, histologic examination of the brains of the treated animals revealed a virtual absence of leukemic infiltration, which was also evident in the liver and kidneys, as compared with untreated leukemic controls (to be published elsewhere). The results presented here are from representative experiments and were repeatedly obtained using the same tumor line as well as P388 leukemia. These findings and their relevance for the increased survival time of treated leukemic mice are currently under investigation in our laboratory. Furthermore, it is interesting to note that selective antimetastatic properties, consisting of the prevention of hematogenous dissemination of cells from solid tumors in mice, have already been observed for *p*-DM-COOK and related aryldimethyltriazenes and have been shown not to be caused by cytotoxic properties of the drugs (13,15,23,24).

The antimetastatic effects of *p*-DM-COOK for leukemic cells also, as suggested by the presently reported experiments, are of interest in view of the possibility of preventing the establishment of leukemic cells in the host's structures, such as those of the CNS, that are not easily accessible to chemotherapy with cytotoxic agents.

REFERENCES

1. Venditti JM. Antitumor activity of DTIC (NSC-45388) in animals. Cancer Treat Rep 60:135–140, 1976.
2. Comis RL. DTIC (NSC-45388) in malignant melanoma: a perspective. Cancer Treat Rep 60:165–176, 1976.
3. Beretta G, Bonadonna G, Bajetta E, et al. Combination chemotherapy with DTIC (NSC-45388) in advanced malignant melanoma, soft tissue sarcoma, and Hodgkin's disease. Cancer Treat Rep 60:205–211, 1976.
4. Slavik M. Clinical studies with DTIC (NSC-45388) in various malignancies. Cancer Treat Rep 60:213–214, 1976.
5. Connors TA, Goddard PM, Merai K, et al. Tumor inhibitory triazenes: structural requirements for an active metabolite. Biochem Pharmacol 25:241–246, 1976.
6. Giraldi T, Nisi C, Connors TA, et al. Preparation and antitumor activity of 1-aryl-3,3-dimethyltriazene derivatives. J Med Chem 20:850–853, 1977.
7. Giraldi T, Goddard PM, Nisi C, et al. Antitumor activity of hydrazones and adducts between aromatic aldehydes and *p*-(3,3-dimethyl-1-triazeno)benzoic acid hydrazide. J Pharm Sci 69:97–98, 1980.
8. Kolar GF, Maurer M, and Wilschutte M. 5-(3-Hydroxymethyl,3-methyl-1-triazeno)imidazole-4-carboxamide is a metabolite of 5-(3,3-dimethyl-1-triazeno)imidazole-4-carboxamide (DIC, DTIC NSC-45388). Cancer Lett 10:235–241, 1980.
9. Hickman JA. Investigation of the mechanism of action of antitumor dimethyltriazenes. Biochimie 60:997–1002, 1978.
10. Gesher A, Hickman JA, Simmonds RJ, et al. α-Hydroxylated derivatives of antitumor dimethyltriazenes. Tetrahedron Lett 50:5041–5044, 1978.
11. Audette RCS, Connors TA, Mandel HG, et al. Studies on the mechanism of action of the tumor inhibitory triazenes. Biochem Pharmacol 22:1855–1864, 1973.
12. Giraldi T, Nisi C, and Sava G. Investigation on the oxidative *N*-demethylation of aryltriazenes in vitro. Biochem Pharmacol 24:1793–1797, 1975.
13. Sava G, Giraldi T, Lassiani L, et al. Mechanism of the antimetastatic action of dimethyltriazenes. Cancer Treat Rep 63:93–98, 1979.
14. ———. Mechanism of the antileukemic action of DTIC and its benzenoid analogue DM-COOK in mice. *In* Proceedings of the Ninth Symposium on the Biological Characterization of Human Cancer, the Control of Tumour Growth and Its Biological Bases. Excerpta Medica, in press.
15. Giraldi T, Sava G, Cuman R, et al. Selectivity of the antimetastatic and cytotoxic effects of 1-*p*-(3,3-dimethyl-1-triazeno)benzoic acid potassium salt, (±)1,2-di(3,5-dioxopiperazin-1-yl)propane and cyclophosphamide in mice bearing Lewis lung carcinoma. Cancer Res 41:2524, 1981.
16. Kolar GF. Synthesis of biologically active triazenes from isoluble diazonium salts. Z Naturforsch 27b:1183–1185, 1972.
17. Lowry OH, Rosebrough NJ, Farr AL, et al. Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275, 1951.
18. Cochin J, and Axelrod J. Biochemical and pharmacological changes in the rat following chronic administration of morphine, nalorphine and normorphine. J Pharmacol Exp Ther 125:105–110, 1958.
19. Kolar GF, and Preussmann R. Validity of a linear Hammet plot for the stability of some carcinogenic 1-aryl-3,3-dimethyltriazenes in an aqueous system. Z Naturforsch 26b:950–953, 1971.
20. Sentiurć M, Schara M, Nemec M, et al. Leukemic L-1210 cell membrane modifications by cholesterol treatment. Studia Biophysica, Berlin 75:13–24, 1979.
21. Giraldi T, Nisi C, and Sava G. Observations on the in vitro oxidative *N*-demethylation of 4-(3,3-dimethyl-1-triazeno)-imidazole-5-carboxamide (DIC, NSC-45388) . Pharmacol Res Commun 8:25–29, 1976.
22. Green AE, and Gesher A. Drug metabolism interactions with anticancer agents in mice. Biochem Pharmacol 29:131–136, 1980.
23. Giraldi T, Houghton PJ, Taylor DM, et al. Antimetastatic action of some triazene derivatives against the Lewis lung carcinoma in mice. Cancer Treat Rep 62:721–725, 1978.
24. Giraldi T, Guarino AM, Nisi C, et al. Antitumor and antimetastatic effects of benzenoid triazenes in mice bearing Lewis lung carcinoma. Pharmacol Res Commun 12:1–11, 1980.
25. Sokal RR, and Rohlf FJ. Single classification analysis of variance. *In* Biometry. San Francisco, CA, W.H. Freeman and Co, 1969, pp 204–252.

Phase II Evaluation of 4′-Epi-doxorubicin in Patients With Advanced Colorectal Carcinoma[1,2]

Richard Michaelson, Nancy Kemeny,* and Charles Young [3,4]

4′-Epi-doxorubicin (4′-epi-DX) is a new doxorubicin derivative that is more active than the parent compound against murine sarcoma virus tumors and Lewis lung carcinoma and may be less toxic. Thirty-five patients with advanced measurable colorectal carcinoma were treated with 4′-epi-DX (85 mg/m^2) every 3 weeks. The major toxic effect was leukopenia < 2000 cells/mm^3 in 28% of the patients. One of 29 patients (3%) had a partial response. At this dose and schedule, 4′-epi-DX has minimal activity in colorectal carcinoma. [Cancer Treat Rep 66:1757–1758, 1982]

4′-Epi-doxorubicin (4′-epi-DX) is a doxorubicin derivative differing from the parent compound by epimerization of the hydroxyl group at the 4′ position (1), which by lowering the affinity of the compound for DNA, results in higher intracellular levels of the drug compared to doxorubicin. 4′-Epi-DX was found to be as active as doxorubicin against Sarcoma 180, Gross' leukemia, L1210 and P388 ascitic leukemias, and 3H/He transplanted mammary carcinoma (1) and to be more active against sarcoma induced by murine sarcoma virus (MSV), metastatic Lewis lung carcinoma, and MS-2 sarcoma (2). Acute and chronic toxicity studies in normal and tumor-bearing mice and humans (2,3) have shown 4′-epi-DX to be less toxic than doxorubicin (2). Myelosuppression, vomiting, alopecia, and stomatitis were less severe than with comparable doses of doxorubicin (3). Two human studies (3,4) also demonstrated less cardiac toxicity, though it is too early to completely evaluate this. In one study, one of six patients with colorectal carcinoma responded. For these reasons, a phase II trial of 4′-epi-DX was initiated in patients with metastatic colorectal carcinoma.

MATERIALS AND METHODS

Thirty-five patients with measurable metastatic colorectal carcinoma were entered in the study. Patients were excluded from the study if they were > 85 years old; if they had a Karnofsky performance status < 50, wbc count < 4000/mm^3, platelet count < 100,000/mm^3, or bilirubin > 2.0 mg/liter; if there was evidence of congestive heart failure; or if they had cardiac ischemia or arrhythmia. Informed consent was obtained from all patients. Patients received a continuous iv infusion of 4′-epi-DX over 20 minutes every 3 weeks. The initial dose of 85 mg/m^2 was increased by 15 mg/m^2 if there was no hematologic toxicity and decreased by 15 mg/m^2 if wbc and platelet count nadirs were < 2000 or < 75,000/mm^3, respectively. A partial response was defined as a > 50% decrease in the sum of the products of two perpendicular diameters of all measurable disease. A minor response was defined as a > 25% decrease in all measurable disease. Stabilization was defined as a < 25% change, while disease progression was defined as a > 25% increase in measurable disease. An adequate trial was at least two doses of chemotherapy with toxicity or disease progression.

The median age of the patients was 56 years (range, 31–80), and the median Karnofsky performance status was 90 (range, 50–100). The median time from diagnosis of metastatic disease to entry in the study was 6 months (range, 1–46). Sites of measurable metastatic disease included liver (74%), lung (31%), abdomen (14%), and lymph node (6%). Twelve patients were previously untreated with chemotherapy or radiation therapy. Of the 23 previously treated patients, 21% had also received

[1]Received Aug 3, 1981; revised May 11, 1982; accepted May 19, 1982.

[2]Supported in part by Public Health Service grant CA-05826 and contract N01-CM-97274 (Division of Cancer Treatment) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services, and by a grant from Farmitalia.

[3]Solid Tumor Service (*R. Michaelson* and *N. Kemeny*) and Developmental Chemotherapy Service (*C. Young*), Department of Medicine, Memorial Sloan-Kettering Cancer Center, New York, NY.

[4]We thank Jane Howard for assistance in record keeping and Pearl Mulraine for secretarial assistance.

**Reprint requests to:* Nancy Kemeny, MD, Solid Tumor Service, Department of Medicine, Memorial Sloan-Kettering Cancer Center, 1275 York Ave, New York, NY 10021.

prior radiotherapy. Prior chemotherapy consisted of 5-FU in 100% of the patients, a nitrosourea in 83%, and phase II agents in 21%. The mean duration of prior therapy was 11 months (range, 2–44).

RESULTS

Twenty-nine of the 35 patients who entered the study received an adequate trial. Of the six inadequately treated patients, four demonstrated marked disease progression before the second dose was administered, one refused a second dose after experiencing marked drug-related thrombocytopenia, and one who had been free of angina for 4 years noted the onset of angina-like chest pain for 1 hour several days after his first dose of the drug, without ECG changes.

The major hematologic toxic effect was leukopenia. Wbc counts $< 3000/mm^3$ were seen in 68% of the patients, and wbc counts $< 2000/mm^3$ were seen in 28%. The wbc count nadir was generally observed 10–14 days after administration of the drug, and full recovery occurred by Day 21 in all but two patients. Two patients who were neutropenic required hospitalization after fever developed. Thrombocytopenia was uncommon; 15% of the patients had a platelet count nadir $< 100,000/mm^3$, and 8% had a nadir $< 50,000/mm^3$.

Alopecia was common, with 85% of the patients having > 50% loss of hair. Nausea and vomiting, which occurred in 46% of the patients, was generally mild and lasted several hours. Moderate fatigue, lasting 3–21 days, was seen in 16 (60%) of the patients. Eight of the 16 patients noted a decrease in fatigue after the drug was discontinued. In four of the patients, symptomatic improvement correlated with response to treatment with other chemotherapy agents. Six (17%) of the patients noted rigors and fever several hours after drug administration. Symptoms of congestive heart failure were not observed, although only one patient received > 250 mg/m^2 of 4′-epi-DX, and this patient had 595 mg/m^2 without cardiac toxicity. None of the patients had mucositis, hepatic dysfunction, or dermatitis.

One of the 29 evaluable patients had a partial response to treatment. This patient had previously been treated with intrahepatic infusion of dactinomycin, 5-FU, and methotrexate. She was treated with 4′-epi-DX following intra-abdominal recurrence and responded with a 75% shrinkage in the size of an abdominal mass. The response lasted 5 months. One patient had disease stabilization by objective criteria but refused a third dose because of marked fatigue and a significant decline in performance status. All other patients had progressive disease.

DISCUSSION

In the treatment of metastatic colorectal carcinoma, doxorubicin as a single agent has been reported to have a partial response rate of 12% (six of 48 patients) in previously untreated patients and of 3% in previously treated patients (5). The true response rate to 4′-epi-DX in a group of previously treated and untreated patients was 3% (one of 29 patients), with a 95% confidence interval of 0%–17%. The one response was in a previously treated patient. It appears that 4′-epi-DX administered in this dose and schedule has a limited role, similar to that of doxorubicin, in the treatment of advanced colorectal carcinoma.

Recently, experiments on human colon tumors transplanted into mice demonstrated no activity with doxorubicin or 4′-epi-DX but definite activity with 4′-deoxydoxorubicin (6). Our observations are consistent with these studies and we will be initiating a study with 4′-deoxydoxorubicin.

REFERENCES

1. Arcamone F, Penco S, Vigavani A, et al. Synthesis and antitumor properties of new glycosides of daunomycinone and adriamycinone. J Med Chem 18:703–707, 1975.
2. Casazza AM, DiMarco A, Bertazzoli C, et al. Antitumor activity, toxicity, and pharmacological properties of 4′-epi-adriamycin. *In* Proceedings of the 10th International Congress of Chemotherapy, Zurich, Switzerland, Sept 1977, vol II, pp 1257–1260.
3. Bonfante V, Villani F, and Bonadonna G. Toxicity and therapeutic activity of 4′-epi-doxorubicin. Cancer Chemother Pharmacol, in press.
4. Schauer P, Wittes R, Gralla R, et al. A phase I trial of 4′-epi-adriamycin. Cancer Clinic Trials 4:433–437, 1981.
5. Frytak S, Moertel CG, Schutt AJ, et al. Adriamycin (NSR-123127) therapy for advanced gastrointestinal cancer. Cancer Chemother Rep 59:405–409, 1975.
6. Giuliani FC, and Kaplan NO. New doxorubicin analogs active against doxorubicin-resistant colon tumor xenografts in the nude mouse. Cancer Res 40:4682–4687, 1980.

Phase I Study of 13-*cis*-Retinoic Acid Toxicity[1,2,3]

P. R. Band,[4,5,*] J. G. Besner,[6] R. Leclaire,[6] C. Girard,[5] G. Diorio,[4] M. Deschamps,[5] M. Gélinas,[5] and D. Larochelle[5]

A phase I study of 13-*cis*-retinoic acid was done in 16 patients with head and neck malignancies using a modified Fibonacci search scheme, with individual doses ranging from 20 to 120 mg/m². Drug doses > 60 mg/m² induced intense headaches, urethritis, desquamative dermatitis, vertigo, and ataxia. The severity of these side effects precludes the use of 13-*cis*-retinoic acid as a potential chemopreventive agent at doses > 60 mg/m². [Cancer Treat Rep 66:1759–1761, 1982]

Retinoids (vitamin A and its synthetic analogs) are essential for the normal growth and differentiation of epithelial tissues and prevent the development of a variety of epithelial tumors in experimental animals (1,2). In man, natural retinoids induce regressions of urinary bladder papillomas, oral leukoplasia, and fibrocystic masses of the breast (2,3). The structural synthetic analog of all-*trans* retinoic acid, 13-*cis*-retinoic acid (13-*cis*-RA), is less toxic than the parent compound (4), is effective in the prevention of lung, bladder, and colon carcinogenesis in rodents (5-7), and produces regressions of basal cell carcinoma in man (8). The clinical phase I study of 13-*cis*-RA described in this report was undertaken to establish the maximal tolerated dose of this drug for its potential use in a chemopreventive setting.

PATIENTS AND METHODS

Sixteen patients (15 males and one female; 33–78 years old) with stage I or II head and neck malignancies were admitted to the study after having fully recovered from the effects of surgery and/or radiation therapy, provided they had normal hemogram, creatinine clearance, and liver function tests. All patients gave informed consent. Baseline studies, repeated at 2-week intervals, included complete medical history and physical examination, complete hemogram, blood chemistries (SMA-12), serum electrolyte levels, and 24-hour creatinine clearance determination. Drug dose was based on body surface area and was given orally as a single daily dose for 3 consecutive months according to the modified Fibonacci search scheme shown in table 1. Each patient remained at his initially assigned dose level until removed from the study. Toxic effects were graded according to the scale shown in table 1.

RESULTS

Treatment characteristics are indicated in table 1. Two patients were treated with 13-*cis*-RA at a dose level of 20 mg/m², six at 35 mg/m², six at 60 mg/m², and one at 90 mg/m². One patient scheduled to receive 60 mg/m² of 13-*cis*-RA inadvertently took twice this amount as his first and only dose. Twelve patients received 13-*cis*-RA for 3 months without dose modification or interruption. Due to severe dryness and painful fissures of the lips, the dose was reduced from 60 to 35 mg/m² in one patient (No. 10). One patient (No. 2) refused treatment after 41 days in the study. Therapy was discontinued in one patient (No. 4) due to the occurrence of metastases, and in three when grade 3 toxic effects supervened. Grade 1 toxic effects occurred in all patients and, except for the severe toxic effects seen in patient 7, represented the only side effects observed at 13-*cis*-RA doses < 60 mg/m². One half of the patients treated with 13-*cis*-RA at doses of 60 mg/m² developed grade 2 toxic effects, whereas in the two patients receiving doses > 60 mg/m², severe toxic effects were encountered.

[1]Received Oct 1, 1981; revised May 10, 1982; accepted May 18, 1982.
[2]A grant-in-aid was supplied by Hoffmann-La Roche Ltd, Canada.
[3]13-*cis*-Retinoic acid was supplied by Hoffmann-La Roche, Nutley, NJ.
[4]Institut du Cancer de Montréal, Quebec, Canada.
[5]Hôpital Notre-Dame, Montreal, Quebec, Canada.
[6]Faculty of Pharmacy, University of Montreal, Quebec, Canada.
**Reprint requests to:* Pierre R. Band, MD, Cancer Control Agency of British Columbia, #700, 686 W Broadway, Vancouver, British Columbia, Canada V5Z 1G1.

TABLE 1.—Characteristics of 13-*cis*-RA treatment and toxic effects

Patient No./sex/ age (yrs)	Treatment duration (days)	13-*cis*-RA dose (mg)				Toxic effect	
		Per m^2	Daily	Cumulative to grade 1 toxicity	Total	Grade*	Type
1/M/76	92	20	30	1710	2760	1	Cheilitis
2/M/58	41	20	40	1040	1640	1	Cheilitis and conjunctivitis
3/M/65	92	35	50	1100	4600	1	Cheilitis, fissured lips, and xerosis
4/F/55	57	35	50	1450	2850	1	Cheilitis and xerosis
5/M/66	92	35	60	900	5520	1	Cheilitis, xerosis, and conjunctivitis
6/M/63	92	35	60	1320	5520	1	Cheilitis and xerosis
7/M/33	92	35	70	660	6440	3	Cheilitis, fissured lips, xerosis, blepharoconjunctivitis, urethritis, and exfoliative dermatitis
8/M/55	92	35	70	1540	6440	1	Cheilitis
9/M/65	92	60	100	800	9200	2	Cheilitis, xerosis, and skin rash
10/M/53	92	60	110	960	5960	1	Cheilitis, fissured lips, and xerosis
11/M/66	92	60	110	1650	10,120	2	Cheilitis, fissured lips, xerosis, conjunctivitis, and skin rash
12/M/37	92	60	120	960	11,040	1	Cheilitis, fissured lips, xerosis, and conjunctivitis
13/M/67	92	60	120	960	11,040	1	Cheilitis, fissured lips, xerosis, and conjunctivitis
14/M/64	92	60	130	1040	11,960	2	Cheilitis, xerosis, conjunctivitis, and increased serum alkaline phosphatase
15/M/49	15	90	160	1280	2400	3	Cheilitis, xerosis, conjunctivitis, fissured lips, skin rash, urethritis, headaches, and desquamative dermatitis
16/M/56	1	120	200	200	200	3	Same as No. 15, vertigo, and ataxia

*Toxicity grade: *grade 1*—cheilitis, xerosis, fissured lips, conjunctivitis, blepharoconjunctivitis; *grade 2*—skin rash, serum alkaline phosphatase increased > 50%; and *grade 3*—headaches, urethritis, and exfoliative dermatitis.

DISCUSSION

The side effects induced by 13-*cis*-RA observed in this study were mostly confined to the skin and mucous membranes. Xerosis and cheilitis were generally mild or moderate in nature and well-controlled with lubrication and topical dermatologic ointments. Skin rashes consisted of maculopapular, sometimes pruritic, eruptions localized over the trunk and limbs; this rash cleared spontaneously despite continuation of treatment at unmodified dose in two patients (Nos. 9 and 11). A mild transient increase in serum alkaline phosphatase was the only laboratory abnormality seen. Except for patient 16, grade 1 toxic effects occurred at an average cumulative dose of 1150 mg (range, 800–1700). Such a dose relationship was not apparent for grade 2 or 3 toxicity. All side effects rapidly disappeared at the cessation of treatment and no chronic toxicity was observed.

The retinoid 13-*cis*-RA has been used mostly in the treatment of various dermatoses and in basal cell carcinoma at an average maximum daily dose of 2 mg/kg (range, 0.5–7.4), with side effects similar to those reported in this study (8–10). Higher doses have been administered to patients with severe keratinizing dermatoses (8,9) or metastatic malignancies (11); the nature of these diseases may require acceptance of the increased toxicity associated with high drug dose.

It must be pointed out, however, that in the context of chemoprevention healthy individuals would be treated and toxic effects should be minimized. The efficacy of 13-*cis*-RA in preventing the progression of preneoplastic lesions to malignancy remains to be clinically proven. In our opinion, the severity of the toxic effects induced by 13-*cis*-RA precludes its use as a potential chemopreventive agent at doses > 60 mg/m^2.

REFERENCES

1. Sporn MB, Dunlop NM, Newton DL, et al. Prevention of chemical carcinogenesis by vitamin A and its synthetic analogs (retinoids). Fed Proc 35:1332–1338, 1976.
2. Bollag W. Retinoids and cancer. Cancer Chemother Pharmacol 3:207–215, 1979.
3. Brocq P, Stora C, and Bernheim L. De l'emploi de la vitamine A dans le traitement des mastoses. Ann Endocrinol 17:193–200, 1956.
4. Hixson EJ, Burdeshaw JA, Denine EP, et al. Comparative subchronic toxicity of all-*trans*- and 13-*cis*-retinoic acid in Sprague-Dawley rats. Toxicol Appl Pharmacol 47:359–365, 1979.
5. Port CD, Sporn MB, and Kaufman DG. Prevention of lung cancer in hamsters by 13-*cis*-retinoic acid. Proc Am Assoc Cancer Res and ASCO 16:21, 1975.
6. Sporn MB, Squire RA, Brown CC, et al. 13-*cis*-retinoic acid inhibition of bladder carcinogenesis in the rat. Science 195:487–489, 1972.
7. Newberne PM, and Suphakarn V. Preventive role of vitamin A in colon carcinogenesis in rats. Cancer 40:2553–2556, 1977.
8. Peck GL, Yoder FW, Olsen TG, et al. Treatment of Darier's disease, lamellar ichthyosis, pityriasis rubra pilaris, cystic acne and basal cell carcinoma with oral 13-*cis*-retinoic acid. Dermatologica 157 (suppl 1):11–12, 1978.
9. Peck GL, and Yoder FW. Treatment of lamellar ichthyosis and other keratinising dermatoses with an oral synthetic retinoid. Lancet 2:1172–1174, 1976.
10. Peck GL, Olsen TG, Yoder FW, et al. Prolonged remissions of cystic and conglobate acne with 13-*cis*-retinoic acid. N Engl J Med 300:329–333, 1979.
11. Cassidy J, Lippman M, Lacroix A, et al. Phase II trial of 13-*cis*-retinoic acid in metastatic breast cancer and other malignancies. Proc Am Assoc Cancer Res and ASCO 22:441, 1981.

High-Dose Medroxyprogesterone Acetate in Advanced Male Breast Cancer[1]

F. Pannuti,* A. Martoni, L. Busutti, M. Gramellini, and E. Piana[2]

Six males with advanced breast cancer were treated with high doses of medroxyprogesterone acetate. In five patients, the treatment induced a partial remission, with a median duration of 7 months. The treatment was well-tolerated. High doses of medroxyprogesterone acetate are effective and well-tolerated therapy for advanced male breast cancer. [Cancer Treat Rep 66:1763–1765, 1982]

Medroxyprogesterone acetate (MAP) is a semisynthetic progestational agent which, employed at high doses im (500, 1500, and 2000 mg/day for 30 days) in the treatment of advanced female breast cancer, induced objective remissions of 43%–45%, with little systemic toxicity (1–3). Previously, MAP used at low doses (< 500 mg/day) stimulated little interest in advanced female breast cancer patients (4–6).

The positive results obtained with high doses prompted us to employ this treatment in male patients with advanced breast cancer as well.

MATERIALS AND METHODS

Six male patients with advanced breast cancer, whose primary clinical characteristics are summarized in table 1, were treated with high doses of MAP. All of the patients had progressive disease. None had undergone antitumor therapy for at least 1 month. None had received hormonal therapy, and one patient had refused orchiectomy. All of the patients had a life expectancy of at least 2 months. Hormone receptor analysis was available only for patient 6, who had an estrogen receptor-positive primary tumor.

Treatment with high doses of MAP consisted of daily administration of the doses reported in table 1 for at least 25–30 days. Four patients received maintenance therapy: patients 1 and 4 were given 1500 and 500 mg im once or twice a week, respectively; patients 5 and 6 were given 1000 mg/day orally. Maintenance therapy was administered to all four patients until tolerance or disease progression.

Objective response was evaluated according to International Union Against Cancer criteria (7). Complete remission (CR) was defined as the disappearance of every tumor lesion for at least 6 months, and partial remission (PR) was defined as a ≥ 50% decrease in the surface area of all measurable tumor lesions or the partial healing of lytic bone lesions for at least 1 month.

RESULTS

As reported in table 1, five of the six patients had objective remission (CR + PR), for a median duration of 7 months. Patient 4 had a CR of pulmonary metastases and of both metastatic supraclavicular and laterocervical lymph nodes. The sixth patient, with liver metastases, had disease progression.

With regard to subjective remission, the three patients with osseous metastases (including the patient showing disease progression) had rapid and significant reduction of pain. The clinical side effects were a gluteal abscess (patient 1), moon face (patients 4 and 6), temporary impotence (patient 4), and erythematous reaction in the truncus which disappeared after the termination of treatment (patient 5).

DISCUSSION

Male breast cancer, like that in females, is considered to be hormone-sensitive. In the advanced stage of the disease, orchiectomy, which has been considered traditionally the first-choice treatment, induced objective remissions in an average of 60% of the patients (range, 45%–87%), with a mean duration of 20 months (8). Recently, using stricter criteria of response evaluation, this treatment induced objective remission in ten of 23 patients (43%) for 17 months (9). Of all the additive

[1]Received Jan 13, 1982; revised Apr 29, 1982; accepted May 25, 1982.

[2]Divisions of Oncology (*F. Pannuti, A. Martoni,* and *E. Piana*) and Radiotherapy (*L. Busutti* and *M. Gramellini*), S. Orsola-M. Malpighi Hospital, Bologna, Italy.

**Reprint requests to:* Dr. Franco Pannuti, Division of Oncology, S. Orsola-M. Malpighi Hospital, Via Albertoni 15, 40138 Bologna, Italy.

TABLE 1.—High doses of MAP in advanced male breast cancer

Patient No.	Age (yrs)	Time from mastectomy (mos)	Disease-free interval (mos)	Previous treatment for metastases	Site of lesion	MAP			Objective response	Duration of remission (mos)	Survival (mos)
						1st course					
						Dose/day (mg)	Total (mg)	Maintenance therapy			
1	42	60	36	Polychemotherapy	Osseous	1500 im	40	Yes	PR	15	22
2	56	10	3	Polychemotherapy	Liver, lung, osseous	1500 im	45	—	Disease progression	—	2
3	63	6	5	—	Osseous, skin	1500 im	38	—	PR	24	27+
4	42	84	48	—	Lung, lymph node	500 im	18	Yes	CR	7	13
5	63	—	—	—	$T_2N_2M_0$	2000 orally	60	Yes	PR	3+	3+
6	48	24	24	—	Lung	2000 orally	60	Yes	PR	6	24+

hormonal therapies, diethylstilbestrol showed objective remission in 21 of 55 patients (10). Sporadic patients were responsive to calusterone therapy after the failure of orchiectomy (11) or 17-hydroxyprogesterone caproate treatment (12). Various investigators recently reported a few cases of advanced male breast cancer responsive to tamoxifen (13–17).

In the past, low doses of MAP were occasionally used in advanced cases of male breast cancer. Even though administration of MAP given at a dose of 100 mg three times a week im failed in two patients (6), oral administration (40–80 mg/day) induced objective remission of the disease in one of five patients when used as a single agent and in two of two when combined with diethylstilbestrol (5). In this study, objective remission was obtained in five of six patients. The remaining patient who had disease progression had diffuse liver metastases, which are notoriously unresponsive to hormonal treatment. The mechanism of antitumor activity of MAP is still unknown. Possibly, MAP acts directly on cancer cells interfering with hormonal receptors (18), whereby the inhibitory action of MAP on the hypothalamo-pituitary-gonadal axis (19) and hypothalamo-pituitary-adrenal-axis (20) would play a primary role. This process would involve a reduction in synthesis of human testicular and adrenal steroids.

The reported results show that high doses of MAP are effective in the treatment of advanced male breast cancer.

If our results are confirmed with more patients, high doses of MAP could be a possible alternative to estrogens and orchiectomy.

REFERENCES

1. Pannuti F, Martoni A, Lenaz GR, et al. Management of advanced breast cancer with medroxyprogesterone acetate (MAP, FI 5837, FI 7401, NSC 26386) in high doses. *In* Functional Explorations in Senology (Colin C, et al, eds). Ghent, Belgium, European Press, 1976, pp 253–265.
2. ———. A possible new approach to the treatment of metastatic breast cancer: massive doses of medroxyprogesterone acetate. Cancer Treat Rep 62:499–504, 1978.
3. Pannuti F, Martoni A, Di Marco AR, et al. Results of a randomized clinical trial of two different massive dosages of medroxyprogesterone acetate (MAP) in the treatment of metastatic breast cancer. Eur J Cancer 15:593–601, 1979.
4. Goldenberger IS. Clinical trial of Δ^1-testololactone (NSC 23759), medroxyprogesterone acetate (NSC 26386) and oxylone acetate (NSC 47438) in advanced female mammary cancer. A report of the Cooperative Breast Cancer Group. Cancer 23:109–112, 1969.
5. Klaassen DJ, Rapp EF, and Hirte WE. Response to medroxyprogesterone acetate (NSC-26386) as secondary hormone therapy for metastatic breast cancer in postmenopausal women. Cancer Treat Rep 60:251–253, 1976.
6. Muggia FM, Cassileth PA, Ochoa M, Jr. et al. Treatment of breast cancer with medroxyprogesterone acetate. Ann Intern Med 68:328–337, 1968.
7. Hayward JL, Carbone PP, Heuson JC, et al. Assessment of response to therapy in advanced breast cancer. Eur J Cancer 18:89–94, 1977.
8. Neifeld JP, Meyskens F, Tormey DC, et al. The role of orchiectomy in the management of advanced male breast cancer. Cancer 37:992–995, 1976.
9. Kraybill WG, Kaufman R, and Kinne D. Treatment of advanced male breast cancer. Cancer 47:2185–2189, 1981.
10. Ribeiro GG. The results of diethylstilboestrol therapy for recurrent and metastatic carcinoma of the male breast. Br J Cancer 33:465–467, 1976.
11. Horn Y, and Roof B. Male breast cancer: two cases with objective regression from calusterone (7*a*, 17*b*-dimethyltestosterone) after failure of orchiectomy. Oncology 33:188–191, 1976.
12. Geller J, Volk H, and Lewin M. Objective remission of metastatic breast carcinoma in a male who received 17-alpha hydroxy progesterone caproate (delalutin). Cancer Chemother Rep 2:77–81, 1961.
13. Abele R, Alberto P, and Megevand R. Letter: Carcinoma of the male breast and oestrogen metabolism. Br Med J 1:1607, 1978.
14. Aisner J, Ross DD, and Wiernik PH. Tamoxifen in advanced male breast cancer. Arch Intern Med 139:480–481, 1979.
15. Cantwell BM, Tong D, Minton M, et al. Tamoxifen and male breast cancer. Lancet 2:582–583, 1978.
16. Hortobagyi GN, DiStefano A, Legha SS, et al. Hormonal therapy with tamoxifen in male breast cancer. Cancer Treat Rep 63:539–541, 1979.
17. Jefferys DB, and Efthimjou J. Carcinoma of the male breast and oestrogen metabolism. Br Med J 1:1697, 1978.
18. Di Marco A. The antitumor activity of 6*a*-methyl 17*a*-acetoxyprogesterone (MAP) in experimental mammary cancer. *In* Role of Medroxyprogesterone in Endocrine-Related Tumors (Iacobelli S, and Di Marco A, eds). New York, NY, Raven Press, 1980, pp 1–20.
19. Pannuti F, Giovannini M, Martoni A, et al. Effects of high dose oral medroxyprogesterone acetate (MAP) on plasma levels of T3, T4, TSH, LH, FSH, PRL, 17 estradiol, testosterone, and aldosterone. IRCS Med Sci 8:764, 1980.
20. Novalk E. Effects of MAP on some endocrine functions of healthy male volunteers. Curr Ther Res 21:320–326, 1977.

Persistent Hypomagnesemia Following Cisplatin Chemotherapy for Testicular Cancer[1]

Richard L. Schilsky,* Audrey Barlock, and Robert F. Ozols[2]

Twenty-nine patients with nonseminomatous germ cell tumors of the testis have been followed for a median of 1.9 years (range, 0.3–3.6) following completion of cisplatin-containing combination chemotherapy. Twenty-two of the 29 patients (76%) became hypomagnesemic during chemotherapy and, of these, 11 (50%) have remained persistently hypomagnesemic for as long as 3 years following discontinuation of cisplatin treatment. In the remaining patients, serum Mg levels returned to normal a median of 0.6 years (range, 0.2–2.1) after completion of chemotherapy. In eight of nine persistently hypomagnesemic patients studied, urinary Mg excretion was inappropriately elevated. Symptoms attributable to hypomagnesemia were rarely reported, although muscle cramps, twitching, or mild tremor were noted by the most severely hypomagnesemic patients. Continued followup of these patients is required to determine the effects of chronic hypomagnesemia in man. [Cancer Treat Rep 66:1767–1769, 1982]

Cisplatin is an inorganic platinum compound that has been of great value in the treatment of disseminated testicular cancer and that shows considerable promise in the treatment of ovarian cancer, carcinoma of the head and neck, and other tumors. Among the toxic effects associated with cisplatin chemotherapy, hypomagnesemia has emerged as a particularly common problem. The great majority of adults and virtually all children receiving cisplatin develop hypomagnesemia at some time during their treatment (1,2). This disorder may be accompanied by hypokalemia and hypocalcemia and may vary in severity from no symptoms to frank tetany. Hypomagnesemia appears to result from a cisplatin-induced defect in renal tubular Mg conservation with subsequent renal Mg wasting (1).

Though the occurrence of cisplatin-induced hypomagnesemia is now frequently recognized, considerable uncertainty exists concerning its natural history and proper management. Since our initial report of cisplatin-induced renal Mg wasting (1), we have had the opportunity to follow a number of patients with nonseminomatous germ cell tumors of the testis who were treated with a cisplatin-containing regimen. This report describes the occurrence and prolonged persistence of hypomagnesemia in such a patient population.

PATIENTS AND METHODS

From 1976 to 1980, patients with advanced testicular cancer referred to the Medicine Branch of the National Cancer Institute (Bethesda, MD) were treated with cisplatin-based combination chemotherapy. The patient characteristics and treatment regimens have been detailed previously (3). Twenty-nine patients have now been without chemotherapy and in complete remission for a median of 1.9 years (range, 0.3–3.6). At the completion of chemotherapy and at each subsequent clinic visit, blood was obtained for measurement of serum electrolytes, calcium, Mg, phosphate, BUN, creatinine, glucose, albumen, and uric acid. Periodically, a 24-hour urine collection was obtained and analyzed for volume, creatinine, and Mg concentrations.

RESULTS

Twenty-two of the 29 patients (76%) became hypomagnesemic (serum Mg < 1.4 mEq/liter) during chemotherapy and have remained persistently hypomagnesemic for a median of 1.3 years following discontinuation of cisplatin. The clinical characteristics of these patients are shown in table 1.

[1]Received Mar 1,1982; revised May 20, 1982; accepted June 1, 1982.
[2]Medicine Branch, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD.

**Reprint requests to:* Richard L. Schilsky, MD, University of Missouri Health Sciences Center, Division of Hematology and Medical Oncology, 807 Stadium Rd, Rm N408, Columbia, MO 65212.

TABLE 1.—Persistent hypomagnesemia following cisplatin chemotherapy in 22 patients

	Median	Range
Total cisplatin administered (mg)	734	450–1100
Time following cisplatin therapy (yrs)	1.9	0.3–3.6
Total duration of hypomagnesemia (yrs)	2	0.3–4
Duration of hypomagnesemia after cisplatin therapy (yrs)	1.3	0.2–3.6
Mg nadir after cisplatin therapy (mEq/liter)	1.08	0.71–1.35
Current serum Mg (mEq/liter)	1.36	0.71–1.80

Though some patients have remained hypomagnesemic for as long as 3.6 years following discontinuation of cisplatin, serum Mg levels in 11 patients have thus far returned to normal. For these patients, serum Mg returned to normal levels at a median of 0.6 years after discontinuation of cisplatin treatment, although hypomagnesemia had persisted in some of the patients for as long as 2.1 years prior to resolution. In patients who presently remain hypomagnesemic, serum Mg levels have now been chronically low for a median of 1.8 years. This prolonged persistence of hypomagnesemia is not clearly related to either the total cisplatin dose administered or the Mg nadir during therapy.

In eight of nine persistently hypomagnesemic patients studied, urinary Mg excretion was inappropriately elevated, with a median urinary Mg level of 6.0 mEq/liter (range, 2.1–10.5). The remaining patient was able to conserve Mg normally (urinary Mg < 0.5 mEq/liter) in the presence of mild hypomagnesemia (serum Mg = 1.20 mEq/liter). Azotemia and hypocalcemia did not occur during the followup period. Median creatinine clearance for the entire group of patients was 83 ml/minute (range, 37–148), and serum calcium levels were normal in all patients. Similarly, symptoms attributable to hypomagnesemia were reported by only two patients. Both patients complained of muscle cramps, twitching, or mild tremor when serum Mg levels decreased to < 1 mEq/liter; however, frank tetany or other central nervous system symptoms did not occur.

DISCUSSION

With the introduction of effective combination chemotherapy, many young men with testicular cancer now achieve sustained disease-free remission and probable cure. As these individuals look forward to the resumption of a normal life-style, the chronic and potential late toxic effects of treatment become increasingly important. The nephrotoxicity associated with cisplatin chemotherapy is manifest not only as a decrease in glomerular filtration rate but also, in many patients, as a specific defect in renal tubular Mg conservation (4,5).

This study clearly demonstrates that in many patients, hypomagnesemia and renal Mg wasting are problems that may persist for years following completion of cisplatin chemotherapy. Though rarely symptomatic in the absence of acute illness, patients may be at risk for the development of tetany during any intervening illness that limits oral intake or promotes excessive gastrointestinal losses of Mg. Further, the effects of chronic hypomagnesemia in man are unknown. Mg is a vital intracellular cation, important for a number of biologic processes. Apart from the effects of acute hypomagnesemia on neuromuscular irritability, Mg deficiency has been implicated as a cause of cardiac arrhythmias, esophageal spasm, and hemolytic anemia (6). Clearly, continued followup of these patients is necessary to assess the potential late effects of chronic hypomagnesemia.

The value of oral Mg supplementation for asymptomatic patients has not been established. Since the primary defect is renal wasting of Mg, it is not clear that Mg supplementation can effectively raise the low serum levels. Of the 22 persistently hypomagnesemic patients in the present study, 13 received continuous oral Mg supplements without a discernible effect on serum Mg levels. Further, of the 11 patients who have recovered from hypomagnesemia, five had received Mg supplements, yet the median duration of hypomagnesemia prior to recovery for these patients (2 years) was, in fact, longer than for the group who returned to normal but never received supplemental Mg (1.5 years). Thus, it would appear that chronic Mg supplementation is not effective in raising serum Mg levels or in hastening the resolution of hypomagnesemia. Whether continuous oral Mg therapy plays any role in preventing the development of symptomatic hypomagnesemia is uncertain.

Symptomatic patients should initially be treated with parenteral $MgSO_4$. These individuals may then require a dietary Mg supplement in an attempt to prevent recurrence of symptoms. A 24-hour urine collection should first be obtained for determination of creatinine clearance and Mg content. The dose of oral Mg prescribed should then be determined based on the extent of urinary losses and after consideration of the fact that the average daily diet contains approximately 25 mEq of Mg, of which only 30%–50% is absorbed (7). It is rarely necessary to raise the serum Mg level to normal since symptoms are uncommon at serum levels > 1.0 mEq/liter.

Cisplatin-induced hypomagnesemia must now be regarded as a chronic and potentially disabling toxic effect in many patients who have been cured of testicular cancer. The long-term effects of hypomagnesemia in this patient population remain to be established. Unfortunately, there are no known ways to prevent cisplatin-induced hypomagnesemia. It will be important, therefore, to evaluate all forthcoming cisplatin analogs in the hope of developing an effective new agent which is nontoxic to the renal tubule.

REFERENCES

1. SCHILSKY RL, and ANDERSON T. Hypomagnesemia and renal magnesium wasting in patients receiving cisplatin. Ann Intern Med 90:929–931, 1979.
2. HAYES FA, GREEN AA, CASPER J, ET AL. Clinical evaluation of sequentially scheduled cisplatin and VM-26 in neuroblastoma: response and toxicity. Cancer 48:1715–1718, 1981.
3. ANDERSON T, JAVADPOUR N, SCHILSKY R, ET AL. Chemotherapy for testicular cancer: current status of the National Cancer Institute combined modality trial. Cancer Treat Rep 69:1687–1692, 1979.
4. MADIAS ME, and HARRINGTON JT. Platinum nephrotoxicity. Am J Med 65:307–314, 1978.
5. BLACHLEY JD, and HILL JB. Renal and electrolyte disturbances associated with cisplatin. Ann Intern Med 95:628–632, 1981.
6. RUDE RK, and SINGER FR. Magnesium deficiency and excess. Ann Rev Med 32:245–259, 1981.
7. WACKER WEC, and PARRISI AF. Magnesium metabolism. N Engl J Med 278:658–663, 712–717, 772–776, 1968.

Tamoxifen in the Treatment of Metastatic Malignant Melanoma[1]

John Wagstaff, Nick Thatcher,* Elaine Rankin, and Derek Crowther[2]

Hormonal influences are thought to be of clinical importance in malignant melanoma (1), and cytoplasmic estrogen receptors in malignant melanoma have been described (2). These considerations led to the use of tamoxifen in the treatment of malignant melanoma, and response rates of 15%–37% have been reported, with response occurring mainly in cutaneous and subcutaneous lesions (2,3). Tamoxifen, a relatively nontoxic, convenient oral agent was prescribed to patients with widely metastatic melanoma who had failed with dactinomycin and dacarbazine chemotherapy.

PATIENTS AND METHODS

Twenty-three consecutive patients who had stage III melanoma (Karnofsky score ≥ 50) with progressing tumor despite chemotherapy with a 5-day regimen of dacarbazine and dactinomycin were entered in the study (table 1). Tamoxifen (10 mg twice daily) was administered until further disease progression occurred. Standard response criteria were used.

RESULTS

Toxicity from tamoxifen was minimal: the drug was not stopped in any patient because of side effects. No patient satisfied the criteria for response: one patient has stable disease and continues to receive tamoxifen at Day 60.

DISCUSSION

The study failed to confirm the efficacy of tamoxifen in malignant melanoma that had been previously reported (2,3). However, the treated patients were a poor-risk group with widespread metastases (both visceral and nonvisceral) and had been previously treated with chemotherapy. Moreover, nine patients received the agent for < 4 weeks. Tamoxifen may have a role in melanoma but not in the poor-risk patients with advanced disease comprising the present study group.

TABLE 1.—Patient characteristics

Characteristic	
No. of males/females	9/14*
Mean age in yrs (range): 53 (29–72)	
No. of patients with systemic metastasis	23†
Duration of treatment (days)	
Median	30
Range	6–118
Median survival from start of treatment (days)	44‡

*5 patients were premenopausal.
†Metastatic pattern: liver, 8; lung, 13; brain, 2; nodal, 18; bone, 11; and cutaneous, 19.
‡5 patients were alive at 2, 3, 6, 9, and 9 mos.

REFERENCES

1. SHAW HM, MILTON GW, FAKAGO G, ET AL. Endocrine influences on survival from malignant melanoma. Cancer 42:669–677, 1978.
2. NESBITT RA, WOODS RL, TATTERSALL MHN, ET AL. Tamoxifen in malignant melanoma. N Engl J Med 29:1241–1242, 1979.
3. MEYSKENS FL, JR. and VOAKES JB. Tamoxifen in metastatic malignant melanoma. Cancer Treat Rep 64:171–173,1980.

[1]Received Oct 7, 1981; revised May 6, 1982; accepted May 25, 1982.
[2]Cancer Research Campaign, Department of Medical Oncology, University of Manchester and Christie Hospital and Holt Radium Institute, England.

Reprint requests to: Dr. Nick Thatcher, Cancer Research Campaign, Department of Medical Oncology, University of Manchester and Christie Hospital and Holt Radium Institute, Wilmslow Rd, Manchester M20 9BX, England.

Phase II Study of Medroxyprogesterone Acetate With Tamoxifen in Advanced Renal Cell Cancer[1,2]

Joachim Z. Fuks,* Joseph Aisner, David A. Van Echo, and Peter H. Wiernik[3]

A small but finite degree of antitumor activity for antiestrogen against renal cell carcinoma has been reported (1–3). Although both progesterone and tamoxifen exhibit antiestrogen activity, these agents may have different mechanisms of effect (4,5), and thus the two hormonal agents could interact to produce greater (or lesser) antitumor response.

Fourteen patients with measurable histologically confirmed renal cell carcinoma not amenable to curative surgery and with Eastern Cooperative Oncology Group performance status ≤ 3 who had not received prior hormonal therapy were entered in the trial.

Patients received tamoxifen at a dose of 10 mg orally 3 times a day and medroxyprogesterone acetate at a dose of 1.0 g/week im for at least 4 weeks. If objective remission or disease stabilization was achieved, therapy was continued until disease progression occurred. Tumor progression was defined as an increase of at least 50% in the size of any measurable lesions or the appearance of new lesions; when tumor progression occurred, therapy was stopped. Pretreatment patient characteristics are outlined in table 1.

TABLE 1.—Pretreatment patient characteristics

Characteristic	No. of patients
Entered/evaluated	14/14
Median age in yrs (range): 57 (25–72)	
Males/females	8/4
Previous treatment	
Nephrectomy alone	6
Chemotherapy alone	1
Nephrectomy and chemotherapy	4
None	3
Metastatic site	
Lung	9
Bone	8
Liver	5

All patients were evaluable for response and toxicity. A total of 121 doses of medroxyprogesterone acetate and 2508 doses of tamoxifen were given to the 14 patients, with a median of six (range, four to 28) and 154 (range, 112–588) doses of medroxyprogesterone acetate and tamoxifen, respectively, per patient. There were no objective responses. Calculated from the first day of treatment, four patients had initial disease stabilization for 4 weeks followed by disease progression, eight patients had disease stabilization for 6–10 weeks, and an additional two patients had disease stabilization for 21 and 28 weeks followed by disease progression. Flushing (three patients), headaches (two), fluid retention (three), and nausea (one) were the only toxic effects observed during this trial. The toxicity was insidious, and its severity was not enhanced on days of medroxyprogesterone acetate administration. Ten patients experienced no side effects.

No antitumor response observed among these 14 patients excludes a 20% level of activity with 90% confidence limits. It is conceivable, however, that the presence of hormone receptors on tumor cells is necessary for hormonal antitumor response or that the reduction of estrogen receptor concentrations by the progestin may have blocked the effectiveness of the antiestrogen. Thus, further studies of hormonal therapy for advanced renal cell carcinoma correlated with estrogen and progesterone receptor concentration of tumor cells may be warranted.

REFERENCES

1. BLOOM HJG. Medroxyprogesterone (Provera) in treatment of metastatic cancer. Br J Cancer 25:250–265, 1971.

[1]Received Jan 8, 1982; revised May 21, 1982; accepted May 25, 1982.
[2]Supported in part by Public Health Service grant CA-32107 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]University of Maryland Cancer Center, University of Maryland Hospital, Baltimore.
**Reprint requests to:* Joachim Z. Fuks, MD, University of Maryland Cancer Center, University of Maryland Hospital, 22 S Greene St, Baltimore, MD 21201.

2. Legha S, and Muggia FM. Antiestrogens in the treatment of cancer. Ann Intern Med 84:751, 1976.
3. Feun LG, Drelichman A, Singhakowinta A, et al. Phase II study of nafoxidine in the therapy for advanced renal carcinoma. Cancer Treat Rep 63:149–150, 1979.
4. Koseki Y, Zava DT, Chamness GC, et al. Estrogen receptor translocation and replenishment by the antiestrogen tamoxifen. Endocrinologie 101:1104–1110, 1977.
5. Hsueh AJW, Peck EJ, and Clark JH. Progesterone antagonism of the estrogen receptor and estrogen induced uterine growth. Nature 254:337–339, 1975.

Phase II Clinical Study of Yoshi 864 in Epithelial Ovarian Carcinoma: A Gynecologic Oncology Group Study[1,2]

M. Slavik,[3] H. Muss,[4] J. A. Blessing,[5] and G. Delgado[6,*]

Yoshi 864 is a busulfan analog active against experimental tumors resistant to mechlorethamine (1). This drug was reported to have some activity in ovarian carcinoma in a broad phase II study at doses causing minimal toxic reactions (2). Therefore, a group-wide phase II study of Yoshi 864 in epithelial ovarian carcinoma, using the same schedule, was undertaken. Thirty-three patients with advanced, recurrent, histologically confirmed epithelial ovarian carcinoma not amenable to further surgery, radiotherapy, or higher priority chemotherapy were evaluated. All patients had measurable disease. Neither ascites nor pleural effusion alone was considered measurable for purposes of this study; sonography was accepted if the lesion was sharply defined during initial examination. All patients had a Gynecologic Oncology Group (GOG) performance status ≤ 3 (median, 1). Patients had to have recovered from the nadir blood cell counts from previous therapy and had to have a granulocyte count $\geq 1500/mm^3$ and a platelet count $\geq 100{,}000/mm^3$ before study entry. A BUN < 25 mg/100 ml, serum creatinine < 1.5 mg/100 ml, bilirubin < 1.0 mg/100 ml, and SGOT < 50 IU/liter were required. Standard response criteria were used (3). Complete response was defined as the disappearance of all gross evidence of disease for at least 1 month, while partial response was a $\geq 50\%$ reduction in the sum of the products of the largest diameter and the perpendicular diameter of all measurable lesions. Progressive disease was defined as a $\geq 50\%$ increase in the sum of these products or the appearance of any new lesions within 3 months of study. Stable disease was defined as a response not meeting criteria for complete or partial remission or progressive disease.

Yoshi 864 was administered by slow iv push at a dose of 2.0 mg/kg/day for 5 days, repeated every 6 weeks. Dose adjustment to 1.5 mg/kg/day was required when significant bone marrow toxicity occurred in the previous course.

Patient characteristics, response to therapy, and toxic effects are summarized in table 1. No objective tumor regressions occurred. Of the 33 patients, six had stable disease, 27 had progressive disease, and 18 received only a single course of the drug because of increasing disease. The most serious toxic effect was thrombocytopenia, reported in 11 patients with a median nadir of $49{,}000$ cells/mm^3 (range, 5000–140,000) and a median occurrence in course 1 (range, 1–2). Leukopenia, with a median nadir of 2700 cells/mm^3 (range, 1300–3900), was reported in 14 patients after median course 1 (range, 1–2). Neurotoxicity, which was manifested as lethargy, drowsiness, disorientation, malaise, or paresthesia, occurred in five patients. The other toxic effects included nausea and vomiting in ten patients and fever and alopecia in individual cases.

We conclude that Yoshi 864 at this dose schedule is not an active drug in the treatment of patients with advanced epithelial ovarian cancer recurring after previous treatment with chemotherapy.

[1]Received Feb 1, 1982; revised May 13, 1982; accepted June 14, 1982.

[2]This study was conducted by the Gynecologic Oncology Group and was supported by Public Health Service grants (CA) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services. The following institutions participated in this study: Georgetown University Hospital, Washington, DC (CA-16938); Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC (CA-21946); Indiana University Medical Center, Indianapolis (CA-21720); University of Minnesota Medical School, Minneapolis (CA-23088); University of Pittsburgh School of Medicine, PA; University of Texas Health Science Center at Dallas (CA-28160); Tufts New England Medical Center, Boston, MA; University of Rochester Medical Center, NY (CA-12482); The Milton S. Hershey School of Medicine of Pennsylvania State University, Hershey (CA-16386); Walter Reed Army Medical Center, Washington, DC (CA-23501); University of North Carolina School of Medicine, Chapel Hill (CA-23073); and the Illinois Cancer Council, Chicago (CA-27806).

[3]Cancer Research and Treatment Center, University of New Mexico, Albuquerque.

[4]Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, NC.

[5]Roswell Park Memorial Institute, Buffalo, NY.

[6]Georgetown University, Washington, DC.

Reprint requests to: Gynecologic Oncology Group Headquarters, Suite 430, 1234 Market St, Philadelphia, PA 19107.

TABLE 1.—Phase II study of Yoshi 864 in advanced epithelial ovarian carcinoma

Age (yrs)	GOG performance status	Previous treatment*	Response to Yoshi 864†	No. of courses of Yoshi 864	Toxic effects
25	0	MELPH, ADR–CTX–CIS	ID	2	Leukopenia (2500 cells/mm^3)
38	1	XRT, ADR–CTX, Megace-tamoxifen	SD	2	Leukopenia (3100 cells/mm^3)
44	0	ADR–CTX	ID	1	Leukopenia (2300 cells/mm^3), lethargy, thrombocytopenia (49,000 cells/mm^3)
44	2	ADR–CTX	ID	1	Leukopenia (3944 cells/mm^3)
49	0	ADR–CTX	ID	2	—
50	0	MELPH, HMM–CTX–ADR–CIS	ID	2	Leukopenia (3900 cells/mm^3), nausea
50	0	MELPH, *C. parvum*, CIS–CTX	ID	1	Disorientation, thrombocytopenia (5000 cells/mm^3), nausea, vomiting
52	1	MELPH, ADR–CTX–CIS	ID	1	Nausea
52	2	ADR–CTX	ID	2	—
53	0	MELPH, P-32, CIS, CTX–HMM, MTX–5-FU	SD	2	Paresthesia, nausea, anemia, leukopenia (2000 cells/mm^3), thrombocytopenia (43,000 cells/mm^3)
53	0	XRT-MELPH, ADR–CTX, CIS	SD	3	Leukopenia (3300 cells/mm^3), thrombocytopenia (98,000 cells/mm^3)
53	2	ADR–CTX, XRT, CIS	ID	1	—
55	1	ADR–CTX-CIS	SD	4	Leukopenia (1300 cells/mm^3), thrombocytopenia (21,000 cells/mm^3), lethargy, fever (101°F)
55	3	ADR–CTX	ID	1	Lethargy, drowsiness
57	1	ADR–CTX–CIS	ID	1	—
58	0	MELPH, ADR–CTX–CIS	SD	3	Nausea
58	1	MELPH, ADR–CTX–CIS, HMM	ID	1	Nausea, vomiting
58	2	MELPH, XRT, CIS	ID	1	Leukopenia (3900 cells/mm^3), thrombocytopenia (35,000 cells/mm^3)
59	0	ADR-CTX, VLB–BLEO–CIS	ID	2	Alopecia, thrombocytopenia (140,000 cells/mm^3)
59	0	XRT, MELPH–HMM	ID	2	Leukopenia (2200 cells/mm^3), thrombocytopenia (57,000 cells/mm^3)
59	1	MELPH, ADR–HMM–CIS	ID	1	Severe anemia
61	0	MELPH, CIS	SD	2	Leukopenia (2300 cells/mm^3), thrombocytopenia (80,000 cells/mm^3)
62	0	XRT, ADR–CTX–CIS, HMM, MTX–5-FU	ID	2	Nausea, thrombocytopenia (10,000 cells/mm^3), hemiparesis
62	0	ADR–CTX	ID	1	—
62	1	ADR–CTX–CIS	ID	1	—
62	2	ADR–CTX–CIS	ID	1	—
63	1	MELPH, HMM–5-FU–CTX, CIS–ADR	ID	1	—
65	0	ADR–CTX–CIS	ID	1	Drowsiness, malaise, fever (101°F)
65	3	MELPH	ID	1	Leukopenia (2900 cells/mm^3)
66	0	XRT, CTX–MTX–5-FU, ADR–CIS, HMM	ID	2	Leukopenia (2700 cells/mm^3), thrombocytopenia (133,000 cells/mm^3)
66	3	XRT, MELPH–5-FU–Megace	ID	1	—
70	3	ADR–CTX–CIS, HMM	ID	1	Nausea
75	1	HMM–CTX–MTX–5-FU, ADR–CTX–CIS, XRT	ID	2	Leukopenia (2900 cells/mm^3)

*MELPH = melphalan; ADR = doxorubicin; CTX = cyclophosphamide; CIS = cisplatin; XRT = radiation therapy; HMM = hexamethylmelamine; *C. parvum* = *Corynebacterium parvum*; MTX = methotrexate; VLB = vinblastine; and BLEO = bleomycin.

†ID = increasing disease; SD = stable disease.

REFERENCES

1. El Merzabani MM, and Sakurai Y. A new alkylating antitumor agent effective on experimental tumors resistant to nitrogen mustard. Gan 56:589–598, 1965.
2. Altman SJ, Metter GE, Nealow TF, et al. Yoshi 864 (1-propanol, 3,3'-iminodi-, dimethanesulfonate [ester], hydrochloride): a phase II study in solid tumors. Cancer Treat Rep 62:389–395, 1978.
3. Slayton RE, Creasman WT, Petty W, et al. A phase II trial of VP-16-213 in the treatment of advanced squamous cell carcinoma of the cervix and adenocarcinoma of the ovary: a Gynecologic Oncology Group study. Cancer Treat Rep 63:2089–2092, 1979.

Phase II Trial of Mitoxantrone in Previously Untreated Patients With Colorectal Adenocarcinoma: A Southwest Oncology Group Study[1,2]

John D. Cowan,[3,*] Daniel D. Von Hoff,[4] Bruce McDonald,[5] Robert W. Talley,[6] Joseph D. McCracken,[3] and Timothy Chen[7]

Mitoxantrone is a new bis anthraquinone that, in preclinical screening, has had activity in a number of animal tumor systems, including Colon 25 and Colon 33 tumors (1). A phase I study of mitoxantrone using a single dose every 3 weeks has shown that the dose-limiting toxicity of the drug is leukopenia (2). Based on these data, the present phase II study was initiated to determine the therapeutic value of mitoxantrone in patients with metastatic colon cancer who had not been previously exposed to cytotoxic chemotherapy.

PATIENTS AND METHODS

Patients with histologically confirmed metastatic adenocarcinoma of the colon, without prior exposure to cytotoxic chemotherapy, were entered in this trial. Eligibility criteria included informed consent, a measurable lesion, life expectancy of at least 10 weeks, performance status ≤ 3 according to the Southwest Oncology Group (SWOG) criteria, wbc count $\geq 4000/\mu l$, platelet count $\geq 100{,}000/\mu l$, serum creatinine ≤ 1.5 mg/dl, and bilirubin ≤ 3 mg/dl.

Patient characteristics are shown in table 1.

On the basis of a phase I study (2), mitoxantrone was administered iv at the initial dose of 12 mg/m^2 over 30 minutes in 150 ml of 5% dextrose in water. The dose was repeated every 3 weeks if the wbc count was $\geq 4000/\mu l$ and the platelet count was $\geq 100{,}000/\mu l$. An adequate trial was considered to be two courses. Dose modifications were encouraged and were based on the wbc and platelet count nadirs. If the wbc count nadir was $\geq 4000/\mu l$ and the platelet count nadir was $\geq 100{,}000/\mu l$, the dose was increased by 10%. If the wbc count nadir was $< 2000/\mu l$ and/or the platelet count nadir was $< 75{,}000/\mu l$, the dose was decreased by 17%.

Standard objective response criteria of SWOG were used.

RESULTS

Thirty-four of 36 patients entered in the study were eligible for toxicity evaluation, with 33 of these being evaluable for response (table 1). Two patients died due to progressive colon cancer during their first 21 days in the study, and one refused further therapy after one course of mitoxantrone. Toxicity data demonstrated that the major toxic effect of mitoxantrone was leukopenia (granulocytopenia). The one neutropenic episode associated with infection resolved without sequelae. Only one of the four patients who had received prior pelvic radiation therapy had a granulocyte count nadir $< 1000/\mu l$ ($900/\mu l$). Nonhematologic toxicity was mild. No cardiac, renal, or hepatic toxic effects were reported, and there were no drug-related deaths.

There were no complete responses, but there was one partial response in the 33 evaluable patients. The partial response occurred in a 71-year-old female with measurable disease limited to the lungs; it lasted > 36 weeks.

DISCUSSION

Our data confirmed that leukopenia is the major toxic effect of mitoxantrone, even in patients not previously exposed to cytotoxic chemotherapy. Although preclinical screening data (1) had suggested that mitoxantrone

[1]Received Feb 17, 1982; revised May 12, 1982; accepted May 26, 1982.
[2]Supported in part by Public Health Service grants CA-22411, CA-22433, CA-04915, CA-12644, CA-14028, CA-03392, CA-03096, CA-27057, CA-28862, CA-13238, CA-04919, CA-16957, CA-12213, CA-20319, CA-26766, CA-03389, CA-12104, and CA-16943 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]Brooke Army Medical Center, San Antonio, TX.
[4]University of Texas Health Science Center at San Antonio.
[5]Ontario Cancer Foundation, Windsor, Canada.
[6]Henry Ford Hospital, Detroit, MI.
[7]Southwest Oncology Group Biostatistical Center, Houston, TX.

[8]We thank Ms. Robbie Fuqua for preparation of the manuscript.

*Reprint requests to: Southwest Oncology Group (SWOG-7940/44), Operations Office, 4450 Medical Dr, San Antonio, TX 78229.

TABLE 1.—Patient characteristics

Characteristic	No. of patients
Median age in yrs (range): 60 (41–86)	
Sex	
Males	17
Females	19
Median performance status (range): 1 (0–3)	
Prior therapy	
Pelvic radiation	4
Chemotherapy or immunotherapy	0
Evaluable for—	
Toxicity	34
Response	33
No. of evaluable courses: 143	
1–3 courses	19
4–6 courses	9
7–13 courses	6
Dose modifications (mg/m^2)	
Increase to—	
13.2	11
14.5	1
20.0	1
Decrease to—	
10	1
8	1
Toxicity	
Hematologic*	
PML count nadir (cells/μl)	
500–999	12
< 500	2
Nonhematologic	
Nausea	9
Alopecia	1
Stomatitis	1
Disease response	
Partial	1
Stable†	15
Progressive	17

*Median wbc count nadir = 3000 cells/μl (range, 1400–6400); median polymorphonuclear leukocyte (PML) count nadir = 1174 cells/μl (range, 378–4736); and median platelet count nadir = 225,000 cells/μl (range, 107,000–500,000).

†Median duration = 16 wks (range, 12–39).

might have a clinically significant effect on colorectal adenocarcinoma, our phase II study identified no such effect. In a human tumor cloning system, mitoxantrone had an antineoplastic effect against a number of human tumor histologies (> 50% decrease in colony formation) but had no activity in the 12 evaluable colon cancers (3). This suggests that the soft-agar human tumor cloning assay of Hamburger and Salmon (4) warrants continued investigation as a screening tool for antitumor agents and as a predictor of response or lack of response in the clinic.

REFERENCES

1. JOHNSON RK, ZEE-CHENG RK-Y, LEE WW, ET AL. Experimental antitumor activity of aminoanthraquinones. Cancer Treat Rep 63:425–439, 1979.
2. VON HOFF DD, POLLARD E, KUHN J, ET AL. Phase I clinical investigation of 1,4-dihydroxy-5-8-bis(((2-((2-hydroxyethyl)amino)ethyl) amino))-9,10 anthracenedione dihydrochloride (NSC 301739), a new anthrocenedione. Cancer Res 40:1516–1518, 1980.
3. VON HOFF DD, COLTMAN CA, JR, and FORSETH B. Activity of mitoxantrone in a human tumor cloning system. Cancer Res 41:1853–1855, 1981.
4. HAMBURGER AW, and SALMON SE. Primary bioassay of human tumor stem cells. Science 197:461–463, 1977.

Yoshi 864 Plus Medroxyprogesterone Acetate in Adenocarcinoma of the Kidney: A Southwest Oncology Group Study[1,2]

Stanley J. Altman,[3] Ronald L. Stephens,[4] and John D. Bonnet[5,*]

Systemic chemotherapy has had little or no effect in palliating disseminated adenocarcinoma of the kidney.

In 21 patients treated with medroxyprogesterone acetate (MPA) parenterally, two responses were noted (1). Bloom (2), reporting primarily on MPA and androgen therapy (the latter for MPA failures), observed an overall objective response rate of 16% in 80 patients who had response lasting from 2 to 35 months; only one of the responses was to androgens.

Yoshi 864 was synthesized in an effort to develop an alkylating agent effective in tumors resistant to mechlorethamine (3). The Central Oncology Group (COG) established a dose schedule of 2 mg/kg/day × 5 by iv push, repeated every 7 weeks (4). In a phase II study (5), there were no objective responses among 11 evaluable patients treated for adenocarcinoma of the kidney. However, in a COG pilot study, three of four patients with adenocarcinoma of the kidney, treated with Yoshi 864 and MPA at a dose of 400 mg/week im, achieved partial responses lasting from 2 to 6 months. Because of the promising pilot data, the Southwest Oncology Group decided to further evaluate this combination.

PATIENTS AND METHODS

Thirty-one adult patients (19 males and 12 females) were studied. Twenty-nine were nephrectomized, and six had had prior chemotherapy or hormonal therapy. Twenty-six were patients considered to be good risk and five were considered to be poor risk, which was defined as having any of the following qualities: age > 65 years; radiotherapy in > 50% of the marrow; or bone marrow metastases. All patients had histologically proven diagnosis and objectively measurable disease. Additional requirements were: wbc count > 4000/mm^3 and platelet count > 100,000/mm^3; BUN < 30 mg/100 ml, creatinine < 1.5 mg/100 mg, and total bilirubin < 4 mg/100 ml; no prior MPA or Yoshi 864; recovery from toxicity of prior treatment; life expectancy of at least 90 days; and written informed consent.

Good-risk and poor-risk patients were given 80 and 60 mg/m^2, respectively, of Yoshi 864 by iv push daily for 5 days and 400 mg/week im of MPA. The course of Yoshi 864 was repeated every 49 days if the wbc count was ≥ 4000/mm^3, and the platelet count was ≥ 100,000/mm^3. Blood cell counts were performed on Days 1, 22, and 50. Blood chemistries were obtained on Day 1 and every 49 days if abnormal or at the termination of study if initially normal. A minimum adequate trial was 7 weeks of treatment and follow-up.

Tumor measurements were recorded in centimeters and consisted of the longest diameter and the widest perpendicular diameter. Liver measurements were made at the xyphoid and 8 cm on either side.

Partial remission was a ≥ 50% decrease in the sum of the products of all diameters of measured lesions and, for liver metastases, a 50% reduction in the sums of measurements below the costal margin, with no increase in any lesion or the appearance of new lesions.

RESULTS

Sufficient data were available for analysis of 30 patients. There were two partial responses (one male, one female) lasting for at least 4 and 16 weeks in patients with lung metastases whose only prior therapy was nephrectomy. Toxicity was minimal (table 1).

[1]Received Feb 25, 1982; revised June 10, 1982; accepted June 16, 1982.

[2]Supported in part by Public Health Service grants CA-13238, CA-12644, CA-22411, CA-21116, CA-03096, CA-27057, CA-03392, CA-22433, CA-12014, and CA-16943 from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services.

[3]LDS Hospital and University of Utah Medical Center, Salt Lake City.

[4]University of Kansas Medical Center, Kansas City.

[5]Scott and White Clinic, Temple, TX.

**Reprint requests to:* The Southwest Oncology Group, Operations Office, Cancer Therapy and Research Center, 4450 Medical Dr, San Antonio, TX 78229.

TABLE 1.—Toxic effects in 30 patients

Toxic effect	Grade	No. of patients
Leukopenia	1 (3000–3999 wbcs/mm^3)	8
	2 (2000–2999 wbcs/mm^3)	5
	3 (1000–1999 wbcs/mm^3)	2
Thrombocytopenia	1 (75,000–99,999 platelets/mm^3)	2
	2 (50,000–74,999 platelets/mm^3)	4
	3 (25,000–49,999 platelets/mm^3)	2
Anemia	1 (Hgb 9–9.9 g/100 ml)	5
	2 (Hgb 7–8.9 g/100 ml)	2
	3 (Hgb 5–6.9 g/100 ml)	2
Nausea	1 (minimal)	1
Diarrhea	1 (minimal)	1
Somnolence	1 (minimal)	1
Fluid retention	1 (minimal)	1
Mucositis	1 (erythema)	1
Decreased libido		1

DISCUSSION

There were brief partial responses in two patients (7%). The patient whose response lasted 4 weeks was removed from the study because of the appearance of symptomatic brain metastases, although his lung lesions were still responding. As used in this study, MPA plus Yoshi 864 is no better than MPA alone.

REFERENCES

1. SAMUELS ML, SULLIVAN P, and HOWE CD. Medroxyprogesterone acetate in treatment of renal cell carcinomas. Cancer 22:525–532, 1968.
2. BLOOM HJG. Hormone-induced and spontaneous regression of metastatic renal cancer. Cancer 32:1066–1071, 1973.
3. EL-MERZABINI MM, and SAKURAI Y. A new alkylating agent effective in experimental tumors resistant to nitrogen mustard. Gan 56:589, 1965.
4. ALTMAN SJ, FLETCHER WS, ANDREWS ND, ET AL. Yoshi 864 NSC-102627 1-propanol,3,3′-iminodi-dimethanesulfonate (ester) hydrochloride: a phase I study. Cancer 35:1145–1157, 1975.
5. ALTMAN SJ, METTER GE, NEALON TF, ET AL. Yoshi 864 (1-propanol, 3,3′-iminodi-dimethanesulfonate [ester], hydrochloride): a phase II study in solid tumors. Cancer Treat Rep 62:389–396, 1978.

Failure of 5-Day Vinblastine Infusion in the Treatment of Patients With Advanced Refractory Breast Cancer[1]

Ian Tannock,[2,*] Charles Erlichman,[2] Danièle Perrault,[2] Ian Quirt,[2] and Michael King[3]

In a recent publication, Yap et al (1) reported a 40% response rate among 30 evaluable patients with advanced breast cancer who had been extensively treated with other drugs. Because of this experience we initiated similar treatment in 17 patients with advanced breast cancer. We observed no responses and considerable toxicity.

All of our patients had advanced symptomatic metastatic disease, and all had received extensive prior therapy (table 1). Nine of the patients had received vincristine, but only two had received prior vinblastine, given as bolus injections with mitomycin. All patients had measurable disease, and all had normal wbc and platelet counts on entry in the study. Abnormal hepatic function did not contraindicate treatment.

Patients received a continuous 5-day iv infusion of vinblastine at starting doses of 1.2–1.8 mg/m^2/day. Courses were given at 3-week intervals, and in the absence of toxicity, the dose was escalated by 0.2 mg/m^2/day in successive courses. All patients received one full 5-day course, and 11 patients received at least two courses (table 1). Treatment was discontinued because of disease progression in 15 of the patients and because of toxic effects in two others. Patients with toxic reactions had a reduction in duration of treatment to 4 or 3 days on second and subsequent courses. Blood cell counts were normal at the time of restarting treatment in all patients except one.

Patients were evaluated at 3-week intervals. No patient responded to treatment. There was, however, considerable myelotoxicity, with one drug-related death from infection during myelosuppression following the initial course of vinblastine. The polymorphonuclear cell count decreased to $< 1000/mm^3$ in nine patients; two of them had platelet counts $< 50{,}000/mm^3$. Toxicity was not clearly related to dose and usually occurred after the first course. One patient experienced a sudden onset of right leg weakness 6 days after the end of her second course of treatment which then gradually improved: this may have been due to drug toxicity but the patient was known to have CNS metastases that were in remission after cranial irradiation.

In our study vinblastine given by infusion to patients with advanced refractory metastatic breast cancer was ineffective and toxic. Our response rate was 0 (95%

TABLE 1.—Characteristics of 17 patients treated with vinblastine infusion

Characteristic	No. of patients
Sites of disease	
Bone	13
Soft tissue (skin, nodes, etc)	16
Viscera (liver, lung)	9
CNS*	2
Eastern Cooperative Oncology Group performance status	
1	3
2	11
3	3
Prior treatment	
Radiation to chest wall	9
Radiation to bone	8
Hormonal therapy or oophorectomy	17
Chemotherapy	
Cyclophosphamide, methotrexate, doxorubicin, and 5-FU	17
Vincristine	9
Vinblastine (bolus) and mitomycin	2
Melphalan, dacarbazine, or chlorambucil	3 (1 of each)
Initial dose of vinblastine (mg/m^2/day)	
1.2–1.3	4
1.4–1.5	10
1.7–1.8	3
No. of courses	
1	6
2	7
3–7	4

*Patients with cranial metastases were included only if they were in remission after cranial irradiation and if they had metastases elsewhere.

[1]Received Feb 25, 1982; revised May 10, 1982; accepted June 16, 1982.

[2]Princess Margaret Hospital and Ontario Cancer Institute, Toronto, Canada.

[3]The Mississauga Hospital, Mississauga, Ontario, Canada.

**Reprint requests to:* Ian F. Tannock, MD, The Princess Margaret Hospital, 500 Sherbourne St, Toronto, Ontario, Canada M4X 1K9.

confidence limits, 0–19%). The reason for the discrepancy between our results and those of Yap et al (1) is unclear. All of our patients had far-advanced and extensively treated disease but this was also true of the M. D. Anderson study: the mean number of prior chemotherapy regimens (2.8; range, 2–4) was less in our study than in that reported by Yap et al (1). We also include all patients entered in the study as evaluable for response, whereas four of 34 were excluded in the latter report. However, this policy does not account for the large difference in response rate between the two series (11 responses among 30 patients vs no responses among 17 patients) ($P < 0.01$, Fisher's exact test).

It is possible that vinblastine given by infusion might be more effective and less toxic when given to patients with metastatic breast cancer earlier in the course of their disease. However, since the treatment required either hospitalization or the use of an infusion pump and there are effective alternatives that can be administered to outpatients, we do not recommend vinblastine by infusion for treatment of metastatic breast cancer.

REFERENCE

1. YAP H-Y, BLUMENSCHEIN GR, KEATING MJ, ET AL. Vinblastine given as a continuous 5-day infusion in the treatment of refractory advanced breast cancer. Cancer Treat Rep 64:279–283, 1980.

Fever Due to 6-Mercaptopurine

Fever can be caused by various drugs, including sulfonamides, iodides, thiouracils, barbiturates, and phenolphthalein-containing laxatives. The knowledge that chemotherapeutic agents can produce fever is crucial, since fever in cancer patients receiving chemotherapy is usually thought to be due to infection, consequently leading to broad spectrum antibiotic treatment. This is even more important in patients receiving aggressive chemotherapy for acute leukemia. Chemotherapeutic agents known to produce fever include bleomycin and cytarabine. 6-Mercaptopurine, an agent used in the treatment of acute leukemia, has to our knowledge been reported to produce fever only once (1).

CASE REPORT

A 24-year-old white female with terminal deoxynucleotidyl transferase-positive acute lymphocytic leukemia received remission induction treatment with doxorubicin, vincristine, and prednisone. She was not in complete remission after 5 weeks and was therefore switched to a combination of cyclophosphamide, cytarabine, and 6-mercaptopurine (100 mg orally daily taken for 3 weeks). One week after starting this combination she developed fever > 39°C. Since her granulocyte count was < 1000/mm^3, several cultures were taken and she started receiving three antibiotics; 3 days later miconazole was added to the regimen. All cultures were negative. The fever did not subside until the day after 6-mercaptopurine was discontinued, when she was still severely granulocytopenic. At this point the possibility of drug-induced fever was suspected. Following bone marrow recovery the patient was found to be in complete remission. Maintenance chemotherapy was initiated and consisted of 30 mg of methotrexate iv once per week and 100 mg of 6-mercaptopurine orally daily. About 1 hour after the first dose of 6-mercaptopurine, the patient developed fever > 39°C that lasted for about 6 hours. Following this episode, no abnormal laboratory findings, including results of liver function tests, could be detected. 6-Mercaptopurine was discontinued and 6-thioguanine was substituted, which was well-tolerated. The patient continued to be afebrile during the course of her maintenance treatment with methotrexate and 6-thioguanine.

Although in granulocytopenic patients fever of unknown origin is usually due to infection, fever due to chemotherapeutic agents may be another possibility.

REFERENCE

1. Sawitsky A, Ruohani J, and Levy RN. Drug fever produced by six-mercaptopurine. Ann Intern Med 61:1116, 1964.

Richard Herrmann
Christian Manegold
Dieter Fritze

Medizinische Universitätsklinik
Bergheimer Str. 58
6900 Heidelberg, W Germany

April 22, 1982

Aminoglutethimide-Induced Thrombocytopenia

Hematologic toxicity secondary to aminoglutethimide, although uncommon, has been reported and is usually manifested as leukopenia[1] (1,2). Isolated thrombocytopenia secondary to this drug has occasionally been mentioned but few clinical details regarding this form of toxicity are available. We describe here a case of isolated thrombocytopenia that occurred after 4 weeks of therapy with aminoglutethimide. It resolved rapidly after discontinuation of the drug and recurred immediately upon reinstitution of drug therapy.

CASE REPORT

The patient, a 58-year-old white female, was hospitalized due to worsening low back pain secondary to breast cancer that was metastatic to the lumbosacral spine. She had recently undergone a course of radiation therapy to this region and had received a brief course of cyclophosphamide, methotrexate, 5-FU, and prednisone chemotherapy without substantial relief of her pain. Four weeks prior to admission, she began receiving 250 mg of aminoglutethimide orally four times per day and 100 mg of hydrocortisone every day in divided doses. On the day of admission (November 24, 1980) a cbc showed a hemoglobin of 12.4/100 ml, a hematocrit of 37%, a platelet count of 220,000/mm^3, and a wbc count of 4200/mm^3. The patient's drug regimen included 50 mg of hydrochlorothiazide given orally twice weekly, guaifenesin (Robitussin-DM) cough syrup as needed, aminoglutethimide, and hydrocortisone, all of which were continued.

[1]Harvey HA. Personal communication, 1981.

Twelve days after admission (December 6, 1980), a cbc showed a hemoglobin of 12.5 g/100 ml, a hematocrit of 36.1%, a wbc count of 3400/mm^3, and a platelet count of 40,000/mm^3. Physical examination showed no petechiae, ecchymoses, or hepatosplenomegaly. Prothrombin and activated partial thromboplastin times were normal. All medications were then discontinued but the patient's platelet count continued to fall over the next 3 days to a nadir of 15,000/mm^3, with no significant change in her hematocrit or chronically borderline low wbc count. The patient refused to have a marrow aspirate performed in any area other than the posterior iliac crest, which had been previously irradiated. On December 6, 1980, an aspirate from that area was obtained but was hypocellular and nondiagnostic.

By 7 days later (December 13, 1980) the platelet count had risen to 155,000/mm^3. Aminoglutethimide and hydrocortisone were restarted since the thrombocytopenia was thought to be related to a possible recent viral illness or to be secondary to the hydrochlorothiazide. No other drugs were administered. A repeat platelet count 2 days later (December 15, 1980) was 50,000/mm^3, prompting discontinuation of the aminoglutethimide. The prothrombin and activated partial thromboplastin times were normal and the peripheral blood film was remarkable only for showing a decrease in the number of platelets. Over the next 9 days the platelet count progressively increased to 250,000/mm^3 and has been normal ever since, even after reinstitution of hydrochlorothiazide.

Hematologic toxicity due to aminoglutethimide has been reported infrequently, although one well-documented case of pancytopenia due to aminoglutethimide has been published (3). Occasional cases of agranulocytosis and/or thrombocytopenia thought to be secondary to this drug have been seen, but in some of these instances the relationship between the drug and the hematologic toxicity has not been clear-cut[1] (1). A recent paper by Asbury et al (2) reports a 4% incidence of thrombocytopenia in a group of 72 women treated with aminoglutethimide. However, no details regarding the thrombocytopenia were given in their paper.

When isolated thrombocytopenia first developed in this patient, its relationship to aminoglutethimide was not recognized, since thrombocytopenia is more commonly associated with the use of the thiazide diuretics. When thrombocytopenia developed rapidly following rechallenge with aminoglutethimide, a cause-and effect relationship was established. The rapidity of onset of thrombocytopenia in our patient is most consistent with a peripheral destructive mechanism, although an isolated immune or toxic suppression of megakaryocytes is also possible. Discontinuation of the drug after both initial therapy and rechallenge resulted in normalization of the platelet count within 7 days. Unfortunately, it was not possible to perform further studies that would have been helpful in elucidating the specific pathophysiologic mechanisms involved.

REFERENCES

1. Santen RJ, Worgul TJ, Samojlik E, et al. A randomized trial comparing surgical adrenalectomy with aminoglutethimide plus hydrocortisone in women with advanced breast cancer. N Engl J Med 305:545–551, 1981
2. Asbury RF, Bakemier RG, Folsch E, et al. Treatment of metastatic breast cancer with aminoglutethimide. Cancer 47:1954–1958, 1981.
3. Lawrence B, Santen RJ, Lipton A, et al. Pancytopenia induced by aminoglutethimide in the treatment of breast cancer. Cancer Treat Rep 62:1581–1583, 1978.

Blair Ardman
Richard Rudders*

Department of Medicine
Hematology/Oncology Division
New England Medical Center Hospital
171 Harrison Ave
Boston, MA 02111

May 24, 1982

*To whom reprint requests should be addressed.

ANNOUNCEMENTS

Roswell Park Memorial Institute Continuing Education Programs

Buffalo, New York, October–December 1982

Roswell Park Memorial Institute is conducting monthly continuing education programs in oncology for community practitioners to disseminate information on the newest methods in the diagnosis and treatment of cancer. Each monthly seminar is organized by a different department of the Institute covering a different clinical specialty. These seminars qualify for Category I credit toward the AMA Physician's Recognition Award, prescribed credit by the American Academy of Family Physicians, and Category 2-D credit by the American Osteopathic Association. There is a $25.00 registration fee for 1-day seminars and a variable fee for longer programs. The seminars are cosponsored by the Cancer Control Program of the Institute and the American Cancer Society, New York State Division and Erie County Unit.

The seminars are scheduled as follows: Cancer in the Elderly - An Interdisciplinary Perspective, *October 7–8, 1982* (Chairperson: Dr. Evan Calkins); Annual Meeting of the New York State Cancer Programs Association, *October 8–9,1982;* Symposium on Dentistry and Cancer, *October 14,1982* (Chairperson: Dr. Norman Schaaf); Computers in Medical Practice, *October 21, 1982* (Chairperson: Fil E. Vanvoris); Lung Cancer: Diagnosis and Therapy, *November 4, 1982* (Chairperson: Dr. Ronald Vincent); Contemporary Issues in Osteoporosis, *November 18, 1982* (Chairperson: Dr. Julian L. Ambrus); Comprehensive Care of the Advanced Cancer Patient, *December 9, 1982* (Chairperson: Dr. Edward Bockstahler).

For further information, contact: Gayle Bersani, RN, Cancer Control Coordinator, Roswell Park Memorial Institute, 666 Elm St, Buffalo, NY 14263 (telephone: 716-845-4406).

Controversies in the Management of Early Breast Cancer

Los Angeles, California, November 17, 1982

This symposium, sponsored by the Hospital of the Good Samaritan, will take place at the Biltmore Hotel in Los Angeles, and will be acceptable for up to 6 hours of Category 1 credit toward CMA and AMA certificates in continuing medical education.

The fee, which includes a luncheon, will be $80 for practicing physicians, $45 for allied health professionals, and $35 for interns, residents, fellows, and retired physicians.

For further information, contact: Bonnie VanWaardenburg, The Hospital of the Good Samaritan, 616 S Witmer St, Los Angeles, CA 90017. Telephone: 213-977-2345.

Second Annual Bristol-Myers Symposium on Nutrition Research

Washington, DC, December 9–10, 1982

The role of nutrition in preventing and treating cancer will be examined by leading nutritional scientists at this symposium. Scheduled to be held at the Shoreham Hotel, the meeting is being organized by the Department of Nutrition Sciences, University of Alabama at Birmingham, and is sponsored by the Bristol-Myers Company and its Mead Johnson & Company subsidiary.

Outstanding scientists from a variety of disciplines will cover a range of topics, including vitamin deficiencies, fragile chromosomes, mononucleosis, lymphoma, nutritional "rescue," and primary chemoprevention with nutrition factors. Dr. Charles E. Butterworth, Jr, and Dr. Martha Hutchinson will cochair the 2-day event.

A major element in the program will be the presentation of the second annual Bristol-Myers Award for Distinguished Achievement in Nutrition Research. The recipient of the $25,000 cash award, selected by an independent panel of physicians and scientists, will be honored at a dinner on December 9.

For further information, contact: Ann D. Wyant or Kathryn R. Bloom, Bristol-Myers Co, 345 Park Ave, New York, NY 10154 (telephone: 212-546-4337).

Nominations For Sixth Annual Bristol-Myers Cancer Research Award

Nominations are now being accepted for the sixth annual Bristol-Myers award for distinguished achievement research. The award winner will be selected by an 11-member panel of judges from cancer research centers in the United States and Europe and will receive $50,000.

Nominations will be accepted from medical schools, free-standing hospitals, and cancer reseach centers until December 15, 1982. Only one nomination from each institution will be accepted.

For forms and further information, contact: Secretary, Awards Committee, Bristol-Myers Co, 345 Park Ave, Rm 43-38, New York, NY 10154.

Second Winter Symposium on Hematologic Malignancies

Snowbird, Utah, February 12–19, 1983

This symposium will feature a multidisciplinary faculty from the University of Arizona Cancer Center. They will review recent diagnostic and therapeutic advances in the lymphomas, leukemias, and multiple myeloma.

For further information, contact: Stephen E. Jones, MD, Section of Hematology and Medical Oncology, University of Arizona Cancer Center, Tucson, AZ 85724.

Cancer Invasion and Metastasis

Houston, Texas, March 1–4, 1983

This conference, the 36th Annual Symposium on Fundamental Cancer Research, will be sponsored by the University of Texas System Cancer Center, M. D. Anderson Hospital and Tumor Institute, and will be held at the Shamrock Hilton Hotel. The meeting will consider recent developments in the biology of tumor metastasis formation and investigate the possible contributions of these developments to cancer treatment. Program topics include tumor cell properties and metastasis, tumor invasion, tumor heterogeneity, host factors and metastasis, and therapy of metastasis. Cochairmen are Drs. Garth L. Nicolson and Luka Milas.

For additional information, contact: Office of Conference Services, Box 18, M. D. Anderson Hospital and Tumor Institute, 6723 Bertner Ave, Houston, TX 77030, or telephone (713) 792-2222.

American Radium Society Meeting

Savannah, Georgia, April 5–9, 1983

The 65th annual meeting of the American Radium Society will be held at the Hyatt Regency Hotel in Savannah, Georgia. *For further information, contact:* Mrs. Sally Polek, Office of the Secretariat, American Radium Society, 925 Chestnut St, Philadelphia, PA 19107 (telephone: 215-574-3179).

Fourth Annual Meeting of the Society for Clinical Trials

St. Louis, Missouri, May 8–11, 1983

The sessions of this meeting will focus on the design, organization, management, and analysis of clinical trials. Topics of interest for contributed papers or poster sessions include but are not restricted to: new methodology and associated problems and controversies in the design, monitoring, and analysis of clinical trials; data banks and data management; group or community clinical trials; clinical trials in specialized clinical areas; clinical trial results and their dissemination; patient compliance (adherence); monitoring for adverse effects or side effects; and ethical issues. Abstracts must be received by January 1, 1983.

For further information, contact: Dr. Christian R. Klimt, Secretary, Society for Clinical Trials, Inc, 600 Wyndhurst Ave, Baltimore, MD 21210.

Fourth International Symposium on Platinum Coordination Complexes in Cancer Chemotherapy

Shelburne, Vermont, June 22–24, 1983

This symposium, sponsored by the Vermont Regional Cancer Center and the Norris Cotton Cancer Center, will address new developments in the biology, chemistry, pharmacodynamics, toxicology, and clinical aspects of platinum and other metal complexes as they relate to cancer therapy. The program is being designed to encourage the efficient exchange of concepts and foster discussion for the development of new concepts in this important area.

The program will consist of six scientific sessions. Each session will include presentations by three invited speakers, the viewing of scientific posters prepared by symposium attendees, and open discussion of the posters.

It is anticipated that 100–150 investigators from North America and Europe will participate, and that the proceedings of the symposium will be formally published. Deadline for poster abstracts is December 31, 1982.

For further information, contact: Joan MacKenzie, Vermont Regional Cancer Center, 1 S Prospect St, Burlington, VT 05401. Telephone: (802) 656–4414.

Thirteenth International Congress of Chemotherapy

Vienna, Austria, August 28–September 2, 1983

The 13th International Congress of Chemotherapy will be held at the KogreBzentrum-Hofburg in Vienna, Austria. The scientific program will consist of main lectures, symposia and workshops, round table sessions, and special paper poster discussions.

The program will include antimicrobial as well as anticancer topics. Proceedings will be preprinted and will be available to all participants at the congress. The deadline for submission of manuscripts (ready for photoprinting) is April 1983; the deadline for announcing papers, including title, authors, and main points of results, is December 1982. The preliminary announcement of intention to present a paper should be communicated as soon as possible.

For further information, contact: Secretariat of the XIIIth International Congress of Chemotherapy, c/o Wiener Medizinische Akademie, Alser StraBe 4, A-1090 Vienna, Austria. Telephone: (0222) 42 13 38. Telex: Chemo-A 13/4743. Cables: Medacad Wien.

Malignant Lymphoma: Current Status and Prospects

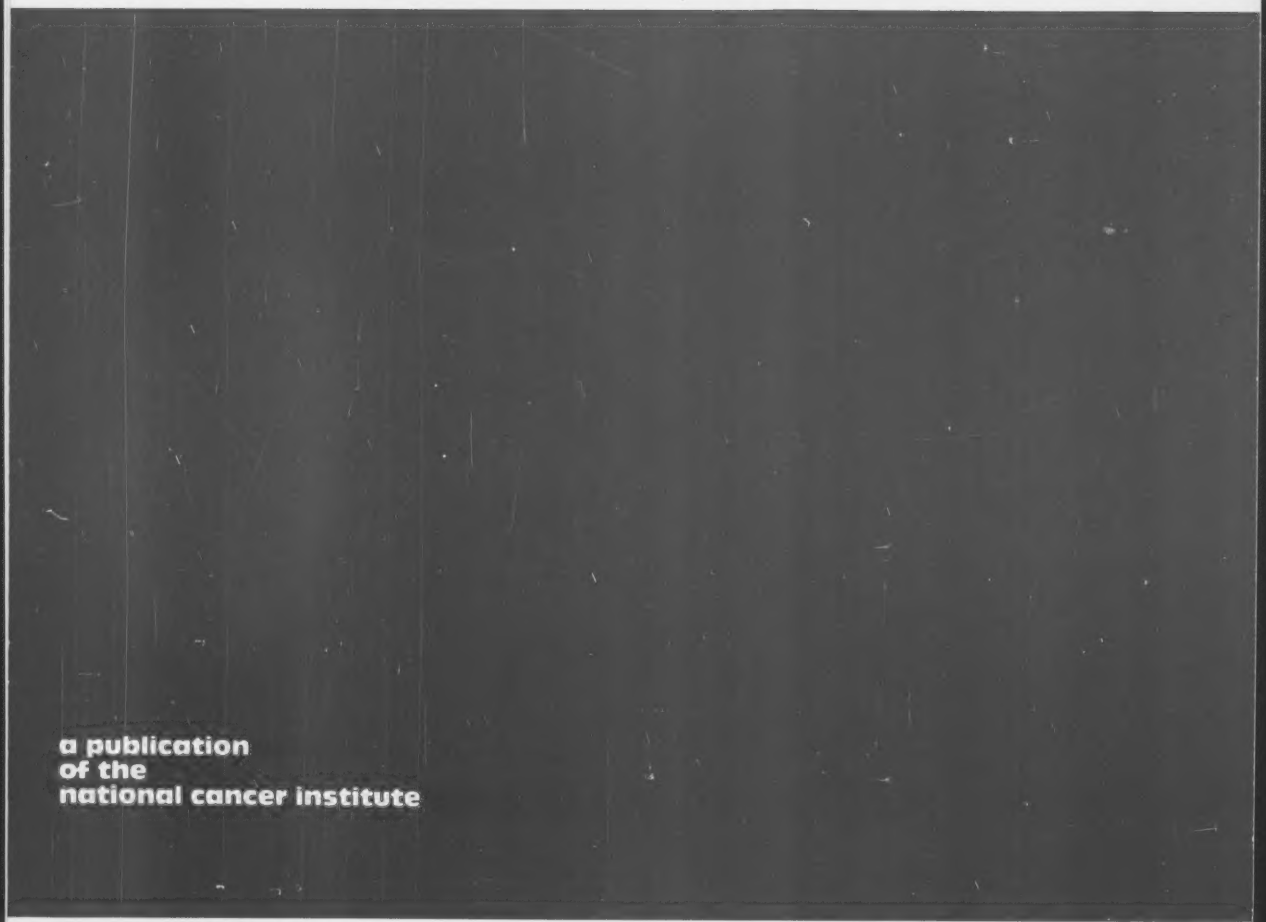
Lugano, Switzerland, June 13–16, 1984

The Second International Conference on Malignant Lymphoma will be held at the Palazzo dei Congressi in Lugano, Switzerland, and will deal with all important approaches in the diagnosis and treatment of Hodgkin's and non-Hodgkin's lymphoma in children and adults.

Most lectures will be given by invited speakers. Selected controversial issues will be discussed by panels of leading experts. The program will include free presentations and a poster session. The deadline for receipt of abstracts is January 15, 1984.

For abstract forms and further information, contact: F. Cavalli, MD, Division of Oncology, Ospedale San Giovanni, 6500 Bellinzona, Switzerland.

*U.S. GOVERNMENT PRINTING OFFICE: 1982 361-140/3 1-3



a publication
of the
national cancer institute

NIH Publication No. 82-22